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SOIL SCIENCE

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THE COLORIMETRIC DETERMINATION OF PHOSPHORUS IN CITRIC ACID EXTRACTS OF SOILS

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One of the important methods of studying soil fertility by chemical means is the citric acid extraction of Dyer (6, 7), with various modifications suitable to special local conditions. The determination of phosphoric acid in these extracts with reasonable accuracy has been a matter of considerable difficulty.

The precipitation of phosphorus as ammonium phosphomolybdate and the titration of the precipitate with sodium hydroxide and nitric acid have, at times, yielded extremely erratic results, even under the most carefully regulated conditions. For approximations of the probable reaction of soils toward phosphatic fertilization over large areas, the results of this method are doubtless of value in the main, but for accurate work on fertilizer experiments and for the determination of the fixing power of soils for phosphoric acid, a more accurate method is imperative.

Solution of the ammonium phosphomolybdate in ammonia, and double precipitation of phosphorus with magnesia mixture, although reliable for moderate or large amounts, is too long and tedious for routine soil analysis and is inaccurate in the case of citric acid extracts which contain small amounts of phosphorus.

Various colorimetric methods were therefore investigated, with the hope of applying one or more of them to the determination of phosphorus in citric acid extracts of soils; the object uppermost in mind was an increase in the accuracy of the results, together with a saving of time and labor, should this be possible, consistent with the reliability desired.

Successful results have been reported by Taylor and Miller (13), Denigès (5), Bell and Doisy (1), together with the modifications by Briggs (2) and Fiske and Subbarow (8). Parker and Fudge (12) studied these methods in relation to their merits for determining phosphorus in soil extracts and concluded that the method of Denigès is approximately five times as sensitive as that of Fiske and Subbarow. Improvements on the Denigès method were recently made by Truog and Meyer (15), who studied the effects of various interfering substances.

All of these methods, however, were intended primarily for use upon solutions relatively free from organic matter and such interfering cations as iron and titanium. Truog and

[1] The author wishes to thank Dr. Francis E. Hance, head of the chemistry department, for coöperation in making possible the pursuance of this research and for suggestions tendered in the preparation of this paper for publication.

Meyer (15) have stated that titanium and ferric iron interfere with the formation of the blue color, whereas ferrous iron does not; they recommend reducing the iron with cadmium in Jones reductors.

More recently, Chapman (4), using the Truog and Meyer method, has shown that the presence of even ferrous iron may be objectionable under certain circumstances; a method for overcoming this difficulty is proposed, but no mention of the effect of titanium is made. The work of Truog and Meyer indicates that concentrations of 20 p.p.m. of this cation interfere seriously.

In the case of colorimetric methods specially intended for the determination of phosphorus in citric acid extracts, extreme rapidity appears to be the object principally sought by investigators. Němec, Laník, and Koppová (11) treat 5 ml. of the extract with sulfuric acid and potassium permanganate and then reduce the excess permanganate with hydrogen peroxide. However, no comment is forthcoming about the effect of iron and titanium, and experiments on extracts of Hawaiian soils have shown that they interfere to such a marked extent as to render the procedure unavailable for use. Moreover, potassium permanganate of the regular C.P. grade carries so much phosphorus that the blank is of the same order of magnitude as the amount of phosphorus in the sample.

Similar objections are valid for the method whereby Zinzadze (17, 18) prepares the solution for analysis; iron was found to interfere.

Warren and Pugh (16) recognized the influence of ferric iron upon the development of the blue color, stating that in certain instances the greenish yellow tint became so pronounced that it was impossible to make comparisons. They developed a procedure involving the destruction of organic matter with sodium permanganate and the removal of iron as the ferrocyanide. Here again, however, the amount of phosphorus present in the reagents is of the same order of magnitude as that in the sample, and inaccurate results are certain to follow in the case of soils low in P_2O_5. Further, the ferrocyanide precipitate is difficult to filter, because of its colloidal condition.

EXPERIMENTAL

Precipitation of iron and titanium by sodium hydroxide was attempted, but it was found impossible to remove all the phosphorus from the precipitate even by double precipitation and repeated washing with hot hydroxide solution containing sulfate. The possibility suggested itself that incomplete recovery might be apparent rather than actual, because of the presence of some substance interfering with the development of the color in the filtrate. But the only substance known to interfere, which might be present in more than negligible amounts in the filtrate from sodium hydroxide precipitation, is quinquevalent vanadium. A determination of the amount of vanadium was therefore made; 0.76 p.p.m. of V_2O_5 were found and tests showed that this amount had no appreciable effect on the formation of the blue color. Chapman's work indicates that many times this amount of vanadium may be present without deleterious influence. It seems, therefore, that incomplete recovery of phosphorus from sodium hydroxide treatment is due to its being held in the precipitate.

Thornton (14) has stated that cupferron, the ammonium salt of nitrosophenylhydroxylamine, is the only known precipitant for titanium that does not coprecipitate orthophosphoric acid. A quantity of cupferron was prepared according to the directions given by Thornton (14, p. 224), and iron, titanium,

vanadium and the like were precipitated directly from the citric acid extracts in the presence of hydrochloric or sulfuric acid. It was found, however, that the excess cupferron was very difficult to destroy. Fusion with magnesium nitrate was unsatisfactory, as was treatment with aqua regia. Gentle ignition in the muffle rendered the residue extremely difficult to redissolve, and treatment with potassium permanganate was inadvisable because of the amount of phosphorus which that reagent carries. Repeated evaporation with nitric and sulfuric acids was successful in destroying the cupferron, but not unless the solution was heated to a temperature at which copious evolution of SO_3 fumes resulted, and under these conditions losses of phosphoric acid occurred by evaporation. In another series of experiments the cupferron was partially destroyed by evaporation with aqua regia and the phosphorus separated by precipitation with ammonia after the addition of aluminum sulfate. This procedure gave low results at times, however, because of incomplete precipitation of the phosphorus.

Electrolytic separation of iron using a mercury cathode

Experiments were then devised upon the electrolytic separation of iron from the solutions, prior to developing the color, using a mercury cathode in which to collect the deposited iron. A very satisfactory separation was obtained, but the titanium remained in solution. Such a neat and convenient removal of the iron, which is the worst interfering cation, resulted, however, that a colorimetric method which was not influenced by titanium was sought.

The method of Truog and Meyer had to be ruled out immediately, since as little as 20 parts of titanium per million result in serious difficulty, and many Hawaiian soils give citric acid extracts in which the concentration of titanium greatly exceeds this amount. Further, the color developed by this method is not stable, increasing in intensity during the first 5 minutes and then gradually fading after 10 or 15 minutes. This instability of color appears to be characteristic of all molybdenum blue methods for phosphorus which call for an excess of reducing agent in the solution.

In the method of Zinzadze (18) no excess reducing agent is present and in consequence the color developed is very stable, remaining undiminished in intensity over a period of days. With this advantage in its favor, the Zinzadze method was definitely adopted when it was found that titanium had little or no influence upon the development of the blue color.

Influence of titanium

In this experiment, a series of solutions containing 2.0 parts of P_2O_5 per million were used, to which varying amounts of titanium had been added. The results of duplicate determinations by the Zinzadze method are shown in table 1.

These results show that titanium may be present in concentrations up to 150 p.p.m. without influence. Although the colors of the solutions con-

taining 200 and 250 p.p.m. were not compared in the colorimeter with standards because of their cloudiness, a rough comparison showed that they were somewhat less intense, because of precipitation of titanium phosphate. It may therefore be concluded that titanium is not harmful unless it reaches a concentration high enough to precipitate some of the phosphate, under which circumstances it will reveal its presence so that the analyst will be forewarned.

Departure from Beer's law in pure solutions

Since it was desired to compare the colors of unknown solutions with standards in the Klett colorimeter, an experiment was conducted to determine how closely Beer's law was followed; this law states that the intensity of color is

TABLE 1

Effect of titanium upon color development

P_2O_5 PRESENT	Ti PRESENT	P_2O_5 FOUND BY ZINZADZE METHOD
p.p.m.	*p.p.m.*	*p.p.m.*
2.0	10	2.05
2.0	10	2.00
2.0	25	2.02
2.0	25	2.04
2.0	50	1.96
2.0	50	1.97
2.0	100	1.93
2.0	100	1.97
2.0	150	2.00
2.0	150	2.01
2.0	200	*
2.0	200	*
2.0	250	*
2.0	250	*

* Intensities not read in the colorimeter; titanium phosphate was precipitated which rendered the solutions cloudy.

proportional to the concentration. Accordingly a series of standards was made up from pure potassium phosphate solutions and the colors compared with that of a solution containing 2.5 p.p.m. of P_2O_5. The average results of duplicate determinations are shown in table 2.

The relation of these results to the theoretical values is shown in figure 1, where milligrams of P_2O_5 present per hundred ml. of solution are plotted against the milligrams per hundred ml. found by colorimetry. The heavy line represents the data, while the light 45° line is the theoretical curve. It is evident that in pure potassium phosphate solutions the deviation from Beer's law is very slight over this range. A very good approximation may be obtained by assuming the theoretical ratio as the basis of calculations, while for the most exact work, the true values may be read from the experimental curve. Since

the standard solution used for comparing the color contained 0.25 mgm. of P_2O_5 per 100 ml., the experimental curve should cross the 45° line at this point, namely, 0.25; the fact that it does not, indicates a small constant error, tending to make the results a trifle high, independent of the deviation from Beer's law.

TABLE 2

Departure from Beer's law in pure phosphate solutions

P_2O_5 PRESENT	P_2O_5 FOUND	P_2O_5 PRESENT	P_2O_5 FOUND
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
0.50	0.58	3.00	3.00
0.60	0.70	3.20	3.32
0.80	0.91	3.40	3.51
1.00	1.03	3.60	3.75
1.20	1.29	3.80	3.90
1.40	1.48	4.00	4.11
1.60	1.68	4.20	4.27
1.80	1.85	4.40	4.39
2.00	2.10	4.60	4.63
2.20	2.22	4.80	4.88
2.40	2.44	5.00	5.02
2.60	2.57	5.20	5.20
2.80	2.86	5.40	5.42

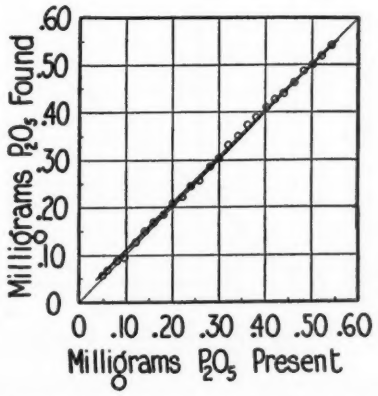

FIG. 1. DEVIATION FROM BEER'S LAW IN PURE POTASSIUM PHOSPHATE SOLUTIONS

Deviation from Beer's law in citric acid extracts

Although the deviation from Beer's law is slight in pure phosphate solutions, the conclusion is not justified that such will be the case in the solutions actually used for analysis and which contain a great many other salts. A citric acid extract which had been prepared for analysis as described in the following was therefore used to determine how closely Beer's law was followed in such a

solution. The procedure was similar to that used for pure phosphate solutions. The standard contained 0.30 mgm. of P_2O_5 per 100 ml., and aliquots of varying size were taken from the unknown. Since the concentration of P_2O_5 in the extract was not known, it was necessary to determine this value in such a way as to avoid error due to deviation from Beer's law. This could have been done by a method of trial and error, in which a series of aliquots of the unknown solution would have been taken until an aliquot volume was found which

TABLE 3

Departure from Beer's law in a citric acid extract

ALIQUOT OF UNKNOWN TAKEN	P_2O_5 PRESENT PER 100 ML.	P_2O_5 FOUND PER 100 ML.
ml.	*mgm.*	*mgm.*
1	0.071	0.078
2	0.142	0.141
3	0.213	0.199
4	0.284	0.288
5	0.355	0.351
10	0.711	0.670
15	1.066	0.944
20	1.422	1.12

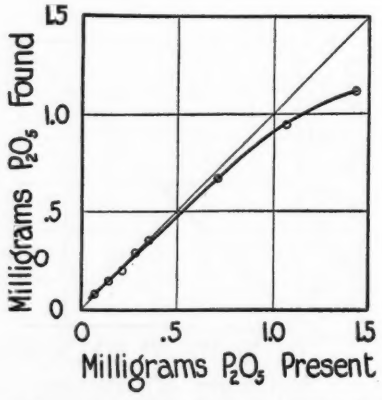

FIG. 2. DEVIATION FROM BEER'S LAW IN A CITRIC ACID EXTRACT

exactly matched the color intensity of the standard and which, of course, would have given a value of unity for the ratio between standard and unknown solution in the colorimeter. It was more convenient, however, to plot the volumes of the various aliquots actually taken in the experiment, against the values of the corresponding colorimeter ratios found and, having drawn the curve representing the data, to read therefrom the volume of the unknown necessary to give a colorimeter ratio of 1. The concentration of the unknown solution

was then calculated from the aliquot volume so determined and the known concentration of the standard. From this value, the actual concentrations of the various solutions were calculated from the volumes of the aliquot portions taken. The resulting data are shown in table 3.

The results are plotted in figure 2. The range of ratios of standard to unknown is somewhat wider than in figure 1, but even so the departure from Beer's law is considerably greater than in the pure solutions; it therefore behooves the analyst to use a standard which is very close to the unknown in color intensity if accuracy is desired.

TABLE 4

Effect of dilution upon color intensity

P_2O_5 PRESENT PER 100 ML.	P_2O_5 INDICATED PER 100 ML.	STRONG STANDARD DILUTED TO 100 ML. BEFORE READING
mgm.	*mgm.*	*ml.*
0.05	0.037	5
0.10	0.084	10
0.15	0.133	15
0.20	0.180	20
0.25	0.225	25
0.30	0.265	30
0.35	0.317	35
0.40	0.375	40
0.45	0.414	45
0.50	0.465	50
0.60	0.561	60
0.70	0.668	70
0.80	0.779	80
0.90	0.892	90

Influence of dilution upon color

Since the color developed by the Zinzadze method is quite stable, as opposed to procedures where an excess of reducing agent is present, the obvious solution to this difficulty is to prepare a wide range of semi-permanent standards; Zinzadze claims that standard solutions retain their full intensity for periods of 7 to 10 days. The writer, however, cannot concur in this matter, having found diminutions of 10 per cent in color intensity after 3 days' standing in the dark.

The inconvenience of making up a large number of standards very frequently, or of taking a second aliquot of the unknown in order to bring it near a single standard, may be avoided by preparing a strong standard and diluting with water till the intensity is close to that of the unknown. Standard color solutions prepared by dilution are not comparable with standards made up in the regular manner, but their values may be determined from a curve. A comparison of diluted standards containing the same amount of P_2O_5 as

standards prepared in the regular manner is shown in table 4. In the left column of the table are shown the actual number of milligram of P_2O_5 present in 100 ml., and the middle column shows the number of milligrams of P_2O_5 per 100 ml. which diluted standards of the same concentrations appeared to contain.

The data of table 4 are plotted in figure 3 and this curve is used to determine the color value of any diluted standard which is made up from the strong one containing 1.00 mgm. of P_2O_5 per 100 ml.

ANALYTICAL

Preparation of citric acid extract for analysis

Apparatus and reagents.—The cell used for the electrolytic separation of iron is a simple modification of the Cain (3) electrolytic cell; it consists of a straight

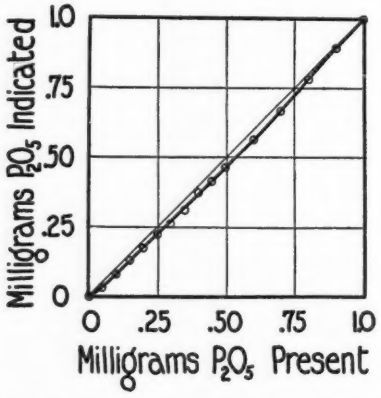

FIG. 3. EFFECT OF DILUTION WITH WATER UPON THE COLOR OF A STANDARD SOLUTION

soil extraction tube having a capacity of about 150 ml. The small glass tube at the bottom of the extraction tube is provided with a short length of rubber tubing and a screw clamp for drawing off the mercury when the electrolysis is completed. The electrodes are supported by a stopper at the top of the extraction tube, the anode being a platinum spiral and the lead to the cathode a short piece of platinum wire sealed in glass, which dips into the mercury at the bottom of the cell, as shown in figure 4. A battery of 20 such cells can be made up simply and cheaply and they are quite satisfactory, although cells provided with glass stop cocks would be considerably more convenient. Cells with rotating anodes, permitting the use of much higher amperages would doubtless be ideal for this purpose, since the time of electrolysis could be enormously reduced.

The mercury used for the cathodes should be purified by washing with hydrochloric acid and subsequently with water before distilling. A small automatic

mercury still (10) may be constructed by anyone with elementary skill in glass blowing and requires no attention other than maintaining the charge of impure mercury and collecting the distillate.

Procedure.—To 100 ml. of citric acid extract, add 50 ml. of concentrated nitric acid, 15 ml. of concentrated hydrochloric acid, and 10 ml. of 20 per cent sulfuric acid free from phosphorus and arsenic. Evaporate slowly till fumes of SO_3 are evolved, being careful not to prolong the evolution of fumes unduly, nor to elevate the temperature excessively, lest phosphoric acid be lost by evaporation. Take up in hot water and boil to insure resolution of any precipitate other than silica which may have formed. Filter into an electrolytic cell provided with a mercury cathode and a platinum spiral anode and electrolyze under a current density at the cathode of 0.03 to 0.05 amperes per

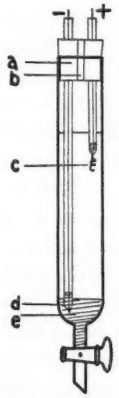

FIG. 4. ELECTROLYTIC CELL FOR SEPARATION OF IRON

a, stopper; *b*, gas vent; *c*, platinum spiral anode; *d*, platinum lead to cathode; *e*, mercury cathode.

square centimeter and a pressure of 6 to 8 volts. After 16 hours, withdraw the mercury from the bottom of the cell and filter the solution into a beaker. Add a few drops of 0.25 per cent hydrogen peroxide in order to reduce any permanganate which may have formed and concentrate to half the original volume in order to insure that the excess hydrogen peroxide, as well as the dissolved hydrogen and oxygen from the electrolysis, are driven off. Transfer to 100-ml. volumetric flasks, take an appropriate aliquot and develop the color by the method of Zinzadze.

Color development by the method of Zinzadze

Reagents.—Molybdenum blue reagent: Heat 50 ml. of phosphorus- and arsenic-free concentrated sulfuric acid to boiling, add 3 gm. of C.P. powdered molybdic acid and boil for 10 or 15 minutes until solution is complete. After

cooling, pour into 50 ml. of water and while still hot add 0.15 gm. of highest purity powdered molybdenum metal as reducing agent; finally, boil the solution for 3 to 5 minutes. After settling 10 to 15 minutes, the blue liquid is decanted from the undissolved metal, cooled, and made up to a volume of 100 ml. It is essential that the reducing power of the reagent be carefully standardized; 2.5 ml. of the solution should bleach exactly 0.2 ml. of normal potassium permanganate. If the reagent is found too weak, it is boiled again with metallic molybdenum, or mixed with the calculated quantity of stronger standardized solution; if it is too strong, it is diluted with the calculated quantity of unreduced 50 per cent sulfuric acid—molybdic acid solution.

Standard phosphate solution: Weigh out 0.1917 gm. of pure recrystallized potassium phosphate (KH_2PO_4) and dissolve in 1 liter. Take a 50-ml. aliquot of this solution and dilute to 500 ml. The later solution contains 0.010 mgm. of P_2O_5 per ml. Transfer 25 ml. of the dilute standard to a 100-ml. volumetric flask, dilute to 90 ml., add 1.4 ml. of molybdenum blue reagent, heat on the steam bath for 30 minutes, cool, and make up to exactly 100 ml. This gives a color standard of convenient strength for use in the colorimeter, but the concentration may be varied to suit individual taste.

The strong solution which was used in determining the dilution curve of figure 3 contained 0.010 mgm. of P_2O_5 per ml.

Determination.—From the solutions of citric acid extract as prepared for analysis, transfer aliquots of from 5 to 50 ml. to 100-ml. volumetric flasks and neutralize to the yellow end point of alpha-dinitrophenol indicator with 10 per cent ammonia solution. Dilute the solutions to 90 ml., add 1.4 ml. of molybdenum blue reagent, heat for 30 minutes on the steam bath, cool, and make up to exactly 100 ml. Employ a sensitive colorimeter for comparison with the standard solution. The ratio between strength of standard and of unknown should be very close to 1 if the highest accuracy is desired; if the standard has been prepared from a stronger solution, in which the color is already developed, by dilution with water as described in the foregoing, a curve similar to figure 3 should be used for correcting the value of the standard used.

Results obtained by employing the method described.—Table 5 shows the degree of reproducibility which may be expected from duplicate determinations. These analyses were made in a regular routine manner without the observance of any elaborate precautions and are representative of some hundreds of duplications obtained at different times and by different analysts, using the method described.

Although good checks are obtained, it still remains to be shown that no proportional errors of any appreciable magnitude are present. For this purpose, a citric acid soil extract was selected for experiment, and varying amounts of P_2O_5 were added to equal aliquots of the extract, in order to learn whether added P_2O_5 might be completely recovered from such solutions.

In table 6, column 1 shows the amounts of P_2O_5 added to 100-ml. aliquots of the extract, and column 3, the amounts found by colorimetric analysis.

In column 2 the amounts calculated for each aliquot from the amounts found in the solutions which received no additions of P_2O_5 are shown, and the per cent deviations of the experimental from the calculated values appear in column 4.

The average deviation is 3.8 per cent and the average recovery, 102.6 per cent. The indicated error in recovery, therefore, is within the experimental

TABLE 5

P_2O_5 soluble in 1 per cent citric acid

PER CENT P_2O_5		PER CENT P_2O_5	
I	II	I	II
0.0345	0.0348	0.0320	0.0320
0.0178	0.0179	0.0369	0.0366
0.0215	0.0248	0.0384	0.0389
0.0386	0.0382	0.0400	0.0403
0.0380	0.0368	0.0417	0.0417
0.0500	0.0540	0.0116	0.0113
0.0352	0.0350	0.0123	0.0119
0.0470	0.0415	0.0227	0.0221
0.0230	0.0220	0.0238	0.0239
0.0240	0.0240	0.0258	0.0254
0.0258	0.0252	0.0278	0.0268

TABLE 6

Recovery of added P_2O_5 from a citric acid extract

P_2O_5 ADDED	P_2O_5 CALCULATED FROM AMOUNT IN UNTREATED EXTRACT	P_2O_5 FOUND	DEVIATION
mgm.	*mgm.*	*mgm.*	*per cent*
0	4.6	4.2	...
0	....	4.9	...
5	9.6	10.1	4.9
10	14.6	14.9	2.0
15	19.6	19.4	1.0
20	24.6	26.4	6.8
25	29.6	31.5	6.0
35	39.6	41.5	2.2
40	44.6	46.3	3.7

error of the method, and evidently no error proportional to the amount of P_2O_5 present appears.

CONCLUSIONS

The stated amounts of hydrochloric and nitric acids are sufficient to destroy all citric acid, as well as organic matter from the soil, in extracts of Hawaiian soils. Soils carrying more organic matter may require further additions.

According to Zinzadze, as much as 700 p.p.m. of SiO_2 has no influence upon the color development and it has been found that separation of most of the silica by dehydration with hot fuming sulfuric acid is a great deal more satisfactory than evaporation to dryness with hydrochloric acid and baking; enough of the silica is eliminated to prevent interference. In the latter method, the large amount of titanium present in Hawaiian soils precipitates a considerable amount of phosphorus which will not redissolve in hydrochloric acid, and for accurate work the residue must be fuzed with sodium carbonate in order to recover the phosphorus.

At the close of the evaporation the sulfuric acid must not be allowed to fume vigorously or at a high temperature, since there is danger of loss of phosphoric acid.

The mercury should be drained from the cells during electrolysis, or immediately thereafter, to prevent resolution of iron.

For the best results, the color should be developed in the standards coincident with developing the color in the unknown solutions.

Special care should be exercised to avoid contamination from foreign sources, such as reagents and glassware. Blanks should be run frequently, preferably with the addition of a known amount of standard phosphate solution just before developing the color, in order to reduce errors due to deviations from Beer's law at the low concentration of the blank.

With the observance of these precautions it should be possible to obtain duplicate analyses differing by no more than 5 per cent at the outside.

SUMMARY

A modification of the Zinzadze colorimetric method for determining phosphorus in citric acid extracts of soils high in iron and titanium has been described.

A method of separating iron from the solutions has been outlined.

It has been shown that as much as 150 parts of titanium per million does not interfere.

The departure from Beer's law in citric acid extracts as contrasted with such departure in pure phosphate solutions has been investigated and reported.

The influence of dilution of the blue color developed by the Zinzadze method has been shown.

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THE RELATION OF CALICHE TO DESERT PLANTS

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Over extended areas in the southwestern United States the soil is impregnated with faintly stratified layers of calcareous hardpan of varying thickness, or filled with hard nodules and amorphous masses of highly calcareous material. In certain localities the nodules have become cemented together into large masses with many lacunae. Associated with these deposits is a finely divided material which contains a small percentage of normal soil constituents and such a high percentage of salts as to be white or nearly white in color. The common type of calcareous hardpan is known in the southwestern states by its Spanish name *caliche*, a word also used in Chile for the analogous deposits of sodium nitrate.

Many types of hardpan have been described and differences have been noted between their location in the soil, their texture, and their composition in the various regions in which they occur. Also, there is general agreement that the manner of formation is not always the same. The position of the material is always closely parallel to the soil surface. The commonest types are found either just below the customary level of tillage or else at the level to which the deepest roots penetrate. Weaver and Crist (8) have described the hardpan of the Great Plains, which lies at a depth of 15 to 36 inches, has a thickness of 8 to 18 inches, and is readily penetrated by roots. This type is stated to disappear when the soil is moist, and its existence is evidently of minor importance in relation to moisture movements in the soil and to root development. Nothing has been done toward a comparative study of the different types of hardpan and little has been written that helps in forming a picture of their geographic distribution. The rather distinctive type which is designated as caliche is known to be of wide occurrence in Arizona, New Mexico, and Sonora, and very similar deposits have been found in adjacent regions.

The observations of the writers have been made chiefly within a 200-mile radius of Tucson, Arizona, and the material of caliche for experimental purposes has been taken within 30 miles of Tucson. In this region caliche is abundant in the soils of outwash plains, or bajadas. The heaviest deposits are found in the lower parts of those bajadas which have been built up solely or largely by outwash from volcanic mountains. Caliche is also found in the soils of limestone and volcanic hills, covering the submerged rock surfaces or filling crevices. In general it is absent from purely granitic soils, from sand, from

the heavy clay, or "adobe," of alluvial flood-plains, and the similar but somewhat coarser alluvial soil of poorly drained basins, or "playas."

The uppermost layers of caliche may be found very close to the surface or at depths as great as 2 or 3 m. They are exposed to view or found very near the surface only in places that have been subjected to recent erosion. The surface of the caliche is undulating and relatively smooth. The thickness of the layers ranges from a few millimeters to 40 cm., but is most commonly between 2 and 5 cm. The lateral extent of the layers is extremely variable, and the continuity of the surface is frequently broken. Approximately the upper half of the surface layer is so hard that it grinds slowly under a file or emery wheel, is very homogeneous in structure, shows a distinct horizontal lamination, and is free of stones and soil particles (pl. 1, fig. 1). The lower portion is somewhat softer, without visible structure, and filled with a greater or less number of stones of various sizes. Beneath the caliche may be found a normal soil, or more often a soil so highly impregnated with salts as to be nearly white, containing pebbles or nodules of material closely similar to the layers of caliche. With increasing depth, successive layers of caliche are found, varying in thickness and frequency, sometimes similar to the surface layer or in other places somewhat softer and less sharply defined. In the larger valleys of southern Arizona, which have been filled with detrital material to great depths, the recurring layers have been found in numerous well digging operations. Under the eastern part of Tucson they have been found in close succession to a depth of 30 m. Meinzer and Kelton (5) have reported cemented layers at a depth of 125 m. in two wells sunk in valley fill at Douglas, Arizona, and definite caliche layers extending for 90 m. to a depth of 230 m. in a well at San Simon, Arizona.

The composition of caliche varies from place to place. Analyses made by the Arizona Agricultural Experiment Station show a content of 55 to 90 per cent calcium carbonate, which is always the predominant constituent. Magnesium carbonate varies from 2 to 5 per cent, and iron, sodium, potassium, and phosphorus are present in small quantities. In some samples calcium silicate and aluminum silicate are present in quantities varying from 10 to 15 per cent. These compounds are generally more abundant in the surface layers and are responsible for their hardness.

Several hypotheses have been advanced in regard to the formation of caliche. All of them take into account the abundance of calcium and the other constituents in the ground water, soils, and common limestone and volcanic rocks of the region. All of them emphasize the important rôle of active evaporation in causing the accumulation of calcareous residues in the soil. Blake (1) laid emphasis on the importance of the upward movement of ground water into levels at which sub-surface evaporation would take place. Lee (4) described localities in the Salt River Valley where caliche was absent from the uppermost 4.5 m. of soil but occurred in the upper layers of the water-bearing gravels which lay just beneath the soil. Forbes (3; 4, p. 110) has attributed the forma-

tion to the penetration of rain water, which dissolves the available salts during its descent and finally precipitates them at the lowest level to which it penetrates. In a recent publication Breazeale and Smith (2) have reviewed all of the hypotheses and their evidences, and have concluded that different groups of factors have been concerned in the formation of the superficial caliche of the bajadas, the deep-seated caliche described by Lee, and the deposits formed on and between rocks on hillsides and mountain slopes. They have also emphasized the importance of plants in removing soil water and causing precipitation at the level of absorbing roots. Breazeale and Smith have accounted for the formation of caliche in bajada soils by reviving the wholly untenable hypothesis of its formation under water in shallow lakes and pools.

The occurrence of successive layers of caliche to a considerable depth is to be accounted for by the gradual upbuilding of the bajadas through deposition of material from the adjacent mountains. Such layers must be regarded as due to descending surface water, since capillarity could not be invoked to raise the ground water, lying from 15 to 25 m. below, if indeed present at all. In soils with a high ground water table or a continuously high water content, it is on the surface that the ascending water causes deposition of salts to take place —the well known "alkali spots" of irrigated desert land.

As maintained by Forbes, the type of caliche found near the surface on the nearly level bajadas of southern Arizona may be regarded as due to descending surface water, limited by climatic conditions in the depth to which it penetrates the soil, and aided by plant roots or soil organisms in the liberation and deposition of its salts. Evidence for the influence of arid conditions on the descending soil water may be had in the rather infrequent occurrence of caliche in the form of small stalactites (pl. 1, fig. 2). These are found where stones or large bodies of caliche are underlaid by small cavities in the ground. The infrequency of the stalactites is solely due to the rare occurrence of such cavities. The hypothesis of Forbes explains all of the observed facts and conditions but still leaves it difficult to account for the absence of caliche from the adobe clay of flood-plains and the similar soil of playas.

INFLUENCE OF CALICHE ON WATER RELATIONS OF THE SOIL

To the student of vegetation, the origin and mode of formation of caliche are not so important as its influence on the physical and chemical properties of the soil and on the development of the root system. The object of the present work has been to investigate some of the physical characteristics of caliche with reference to the movements and availability of water in the soil, and to determine the influence exerted on the growth of a common desert shrub by varying quantities of the material of which caliche is composed.

The degree of insolubility of caliche in water is indicated by the high percentage of calcium carbonate which it contains and by the silicates which are present in the harder types. The presence in soil water of dissolved carbon dioxide, derived from the atmosphere or from plant roots, greatly increases the solubility of caliche under natural conditions.

The structure of the stratified surface layers of caliche is of such density as to provide a very small volume of air space replacable by water on wetting. Seven pieces of air-dried caliche weighing between 100 and 200 gm. were immersed in water for 48 hours. They were then removed from the water, blotted, and allowed to dry on the surface. The percentages of water which they contained, based on the dry weight, were 4.7, 6.5, 3.2, 3.7, 3.0, 4.8, 5.4. These percentages indicate a very low water content as compared with soils. It is evident that caliche is of no importance as a reservoir of moisture, and that it does not have a great enough water capacity to remove any considerable quantity of water from the adjacent soil by capillarity and to hold it where it is not available to plants. The soft type of caliche is much more porous, and similar determinations of its maximum water content have shown it to vary from 12.9 to 17.3 per cent, amounts which are comparable to those found in soils.

When the edge of a dry piece of hard caliche is placed in water it is possible for a short time to observe the wetting by the darkening of the color. The advancing edge of the moist zone soon becomes indistinct, however, even when a dye is used, so that it is not possible to measure easily the time required for the wetting of the entire piece. Neither is the advance of water on the surface an accurate indication of its advance in the interior.

The influence of a single layer of hard caliche on the capillary rise of water in a column of soil was determined by the following experiment. Two glass cylinders were selected, 6.5 cm. in diameter and 15 cm. high. A piece of caliche averaging 1 cm. in thickness was turned down on an emery wheel to the inside diameter of the cylinders and was cemented into one of them 8 cm. from the bottom. A fine clay soil in air-dry condition was then tamped into the cylinder above and below the caliche and the second cylinder was filled in the same manner with soil only. The two cylinders were then placed in a dish with water 2 cm. deep. The experiment was started at noon on May 19. By 2 p.m. of the same day water had reached the top of the control cylinder and the bottom of the caliche layer. At 2 p.m. on May 20, water had penetrated the caliche on one side of the cylinder to a distance of 7 mm. At 4 p.m. on May 21 it had penetrated the entire layer, or at least reached the entire circumference of the cylinder, to an average distance of 1.8 cm. The slow rise of water was measured on successive days and had reached 4.5 cm. above the layer on May 27. The free rise of water to the 8-cm. level in both cylinders required 1 hr. 45 min., whereas the rise above the layer of caliche was 4.5 cm. in 7 days. A repetition of the experiment with another piece of caliche 17 mm. thick and apparently of a somewhat more porous character gave similar results, with movement about 10 per cent greater than in the first case.

In order to test the downward movement of water through caliche an experiment was performed with a cylinder arranged precisely as just described, with room left at the top for placing water to a depth of 1 cm. As water penetrated the soil the amount was renewed to the original level. On the seventh

day the water was poured off the surface, 2 cm. of soil were removed from the top and discarded, and determinations were made of the moisture content of the soil above the caliche layer and of that below it. On the basis of dry weight, the percentages were respectively 34.8 and 18.8. These experiments indicate that even a thin layer of caliche constitutes a serious obstacle to the movement of water in the amounts and at the rates which are possible in free soil.

Edith B. Shreve (7) has shown that a caliche layer may act as a semi-permeable membrane, and it is possible that relatively thin layers with adjacent bodies of soil may play an important part in the movement of water under conditions where soil solutions of different osmotic value exist on the two sides of the caliche. Such movements might be great enough in critical seasons to be of importance to plants.

When the soft type of caliche is reduced to a coarse powder it possesses a mechanical composition close to that of a fine alluvial clay. A comparison of the capillary rise of water in this material and in "adobe" clay was made in the following manner. Tubes 1 cm. in diameter were filled (*a*) with adobe clay, (*b*) with powdered caliche, (*c*) with alternating layers of the two, 10 cm. thick, (*d*) with alternating layers of clay 5 cm. thick and of powdered caliche 1 cm. thick. The capillary rise of water in the four tubes proceeded at nearly the same rate, and in 12 hours reached levels varying from 29 to 30 cm. above the level from which water was drawn. It appears, therefore, that a wide difference exists between the influence exerted on the movement of soil water by layers of hard caliche and by the soft masses which are frequent at lower levels and are easily reduced to the form used in this experiment. Considerable importance attaches to the rate at which water is lost by evaporation from the smooth surface of hard caliche, both in the rare cases in which it is exposed at the soil surface and in the commoner cases in which it is overlaid by a thin and very dry body of soil.

In order to give a basis for comparison of the evaporation from caliche and from the surface of the structurally analogous porous cup atmometer the following experiment was performed. An atmometer was mounted on a glass tube with attached burette. A funnel 9 cm. in diameter was mounted in the same manner. A piece of hard caliche 3 cm. thick was turned so as to fit closely into the funnel, with its upper surface flush with the edge, and was then cemented into place. Water was introduced and a burette attached.

The two evaporating surfaces were placed side by side in the laboratory and the losses read from the burettes. The results, showh in table 1, indicate that the loss from comparable areas was about one-fifth as great from the caliche as from the atmometer. An effort to obtain readings at a higher rate of evaporation resulted in the breaking of the capillary columns of water in the caliche and the appearance of bubbles beneath it.

A further experiment was carried out to determine the rate of evaporation under more natural conditions, using thinner layers of caliche to which water

was supplied by a moist soil. Six pieces of caliche, varying from 5 mm. to 3 cm. in thickness, were turned out to circular form with a diameter of 6 cm. Each of the disks was sealed into a can 6 cm. in diameter and 7 cm. deep, together with adobe clay after the manner shown in fig. 1. The cans were perforated in the bottom. A layer of filter paper was placed in the bottom of each can, the desired amount of air-dry soil was tamped in, the disks were cemented in place. In three cans the caliche disk was placed at the top, while in the other three more soil was tamped in on top of the disks. The cans were set in a pan

TABLE 1

Evaporation from porous cup atmometer and from disk of caliche 9 cm. in diameter and 3 cm. thick

Readings taken in shade and still air of laboratory. Figures indicate losses per 100 sq. cm. per hour.

	ATMOMETER	CALICHE
Apr. 21, 10.00 p.m.	...	...
Apr. 22, 1.00 p.m.	1.5	.30
Apr. 23, 12.00 noon	1.6	.30
Apr. 23, 1.00 p.m.	1.7	.24
Apr. 24, 12.00 noon	1.6	.31
Apr. 24, 2.00 p.m.	2.0	.25
Apr. 24, 9.00 p.m.	2.0	.35
Apr. 24, 11.00 p.m.	2.0	.41
Apr. 25, 12.00 noon	1.7	.31

TABLE 2

Amounts of water lost by caliche lying in or above moist soil, and moisture content of caliche and soil after 12 days

The numbers refer to the experimental arrangement shown in fig. 1. Evaporation in grams, moisture contents in percentages on dry weight.

	1	2	3	4	5	6
Total evaporation in 12 days	17.3	19.0	14.8	40.3	50.2	35.3
Final moisture content of caliche disks	10.0	7.7	5.4	3.2	3.0	4.8
Final moisture content of soil below caliche	21.5	24.0	23.2	8.3	8.5	12.2
Final moisture content of soil above caliche	....	....	....	4.6	4.0	3.5

of water, 1 cm. deep, for 10 days to allow both soil and caliche to reach their maximum water content. They were then removed, dried, and set into shallow tin lids, 7 cm. in diameter, containing hot paraffine, in order to seal the perforations. The cans were kept indoors and weighed daily from April 25 to May 4 and finally on May 7. At the close of the weighings, the cans were opened, and determinations were made of the moisture content of each piece of caliche and of each of the 10 separate bodies of soil. The resulting data are shown in table 2.

In the cans numbered 1, 2, and 3 the caliche layers were placed on the surface. The amounts of evaporation were less from these cans than from the ones with soil exposed at the surface. The amounts of evaporation from cans 1, 2, and 3 did not differ by amounts proportional to the relative thickness of the three layers, but were of the same general order of magnitude. The percentages of moisture remaining in the disks at the close of the experiment were not proportional to the thickness of the disks, although the 3-cm. disk in can 1 contained the highest amount. Although care was taken to select disks which appeared to have uniform texture it is obvious that differences existed in the minute structure of the different ones. Any number of samples of caliche may be expected to differ in texture, and consequently in their water content and rate of evaporation. The bodies of soil beneath the caliche disks showed very different percentages of residual moisture. In the three cans with caliche on the surface (1, 2, and 3) the percentages of soil moisture remained high (21.5 per cent, 24.0 per cent, 23.2 per cent). In the cans containing disks which were overlaid by soil (4, 5, 6) the three exposed bodies of soil were naturally reduced to low percentages, and the three bodies lying be-

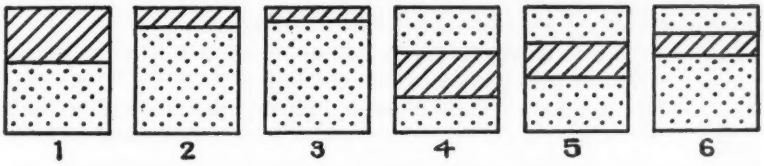

FIG. 1. ARRANGEMENT OF CALICHE IN CANS OF SOIL USED FOR DETERMINING RATE OF EVAPORATION

neath the caliche were also reduced to percentages much lower than those reached by the bodies of soil which were covered by disks which had no soil above them. In terms of figure 1 the moisture content of the lower soil bodies in cans 4, 5, and 6 was lower than in cans 1, 2, and 3. This may be taken to indicate that caliche will convey water more rapidly from the soil beneath it to soil above it than it will from subjacent soil to the atmosphere. The explanation of this may lie in the action of caliche as a semi-permeable membrane.

INFLUENCE OF CALICHE ON PLANTS

The presence of layers of caliche in the soil is of importance to plants from a purely physical standpoint through the mechanical interference with root development and through the modification of the normal movements of water in the soil.

From a chemical standpoint the presence of the softer type of caliche serves to maintain a high concentration in the soil solution. It is not the primary agent in this connection, since caliche is itself due to the prolonged accumulation of salts, and forms in the first instance only in soils so situated topographi-

cally as to become charged with salts leached from the higher levels of the same drainage system.

The interference with roots is easily demonstrated by excavations, in which roots are found running horizontally for as much as 3 to 5 m. along the surface of extended layers of caliche. After penetrating cracks in the first layer the root may be found running horizontally again along the surface of the second layer, not infrequently in the opposite direction to that which it first traversed. In localities on the bajada of the Tucson Mountains with from four to seven well-defined layers of caliche, the roots of *Acacia paucispina* have been found at a depth of 2.5 m., underlaid by poorly stratified and relatively soft caliche.

Several experiments have been made to determine whether roots etch the surface of caliche or penetrate the layers by means of acid excretions. Small pieces of caliche were smoothed on one side, given a high polish, and cemented into flower pots. Corn, beans, cotton, and creosote bush (*Larrea*) were planted in the pots and allowed to remain from 3 to 20 months. No evidences of root etching were found. Larger plants of *Larrea* were raised in pots with unpolished pieces of caliche sealed in place near the bottom, and were allowed to remain for 2 years under greenhouse conditions. There was a large development of roots but no penetration of the caliche layers.

With reference to water movements caliche is a distinct deterrent to the penetration of water from the surface to deeper layers of the soil. When water has finally reached the lower levels, however, the hard layers of the stratified type are very effective in its retention. Field evidence indicates that between favorable and unfavorable functions the balance is in favor of the latter, and that the moisture conditions of the soil would be more favorable to plants without caliche than with it. When the obstruction to root development is taken in conjunction with the influences on soil-water movement, a strong case is developed against caliche as a soil constituent.

Some evidence of the rôle of caliche in relation to plant development may be obtained in the Tucson region, where there are extensive areas both with and without it. On the nearly level outwash plains where caliche is well developed the predominant plant is the creosote bush (*Larrea tridentata* = *Covillea tridentata*, Zygophyllaceae), an evergreen shrub. Perennial plants of secondary abundance in caliche areas are *Acacia paucispina*, *Krameria glandulosa*, *Prosopis velutina*, and several species of cacti. The depth of the soil overlying the caliche can be predicted with a fair degree of accuracy by the density of the stands of *Larrea* and by the height of the bushes, which varies from 50 and 75 cm. in the most shallow soils to 100 and 200 cm. in deep soil. In the Tucson region *Larrea* is infrequent or absent in areas where caliche is not found, although this is not true of it in other parts of its geographical range. When it occurs on soils free of caliche it attains the greater height just mentioned. branches more freely, and bears heavier foliage. On areas free of caliche there is also a heavier stand of *Acacia*, *Parkinsonia*, *Olneya*, *Prosopis*, and other woody perennials. Areas of *Larrea* in Cochise County, Arizona, at an eleva-

tion of 4,000 feet (1,220 m.), with an approximate rainfall of 14 inches (355 mm.), but with soil heavily impregnated with caliche 15 to 25 cm. from the surface, have been compared with areas in the District of Altar in Sonora, Mexico, at an elevation of 1,600 feet (488 m.), with an annual rainfall of 9 inches (228 mm.), but on soil free of caliche. In spite of the higher rainfall in Arizona, the stand of *Larrea* is much heavier and the plants much taller in Sonora. It is apparent from these comparisons that the low and open character of the vegetation, associated with desert conditions and commonly attributed to inadequate water supply, is due in many parts of Arizona and adjacent states to the influence of caliche in restricting root development, reducing the penetration of water, and assisting in the maintenance of a high salt content in the soil solution.

The soft type of caliche has no such effects on the water relations of the soil and the development of the root system as those that have been ascribed to the hard type. Many localities have been examined in which the amount of soft material was greatly in excess of the hard layers. Water movements and root distribution in the thick beds of soft material are more nearly what would be found in a normal soil. The fact that the hard and soft types are almost invariably associated, even when the latter is by far the more abundant, renders it difficult to obtain field evidence on the influences which the soft type may exert on the growth of plants. In order to obtain experimental evidence in soils highly impregnated with soft caliche without having to deal with the known effects of the hard layers, a series of cultures of *Larrea* was arranged and allowed to run for 20 months. Three widely different soils were selected: adobe clay, a granitic loam, and sand. Each of these was used pure and also with varying amounts of finely divided caliche. The amounts of caliche were 10, 25, 50, 75, and 90 per cent. The six soils were made up in duplicate for each of the three types, making 36 cultures, to which were added two pure caliche units. The soils were placed in large carbide cans 12 inches (30 cm.) in diameter and 22 inches (56 cm.) deep. Three plants of Larrea between 5 and 10 cm. high were set in each can in August, 1927. Some replanting was done in September, 1927, to replace plants which died as result of removal. The cans were placed in a lath shelter and were watered lightly once a week except in the winter and summer rainy periods. Out of the 114 plants there were 96 survivals at the termination of the experiment in April, 1929; of the 18 deaths 11 were in cultures with 90 per cent caliche and 10 per cent soil, and one was in the pure caliche.

In table 3 are shown the heights and dry weights of the plants reared under the 19 cultural conditions. Because of the uniform size of the plants at the beginning of the series and the length of time given them for growth, no allowance has been made for the small differences existing at the start. The height figures are averages of the six plants raised under the same conditions, with only two to five plants surviving in six cases. The dry weights are the average for single cultural conditions, with the same limitations due to incompleteness of the series at the close.

With respect to growth in height it will be seen in table 3 that the poorest growth, 20 cm., was made by the plants in pure caliche. In each of the cultures in which 10 per cent of sand, loam or clay was mixed with caliche the growth was from two to two and a half times as great. In the sand series the growth in height in pure sand was not so great as in the mixture 10 per cent sand + 90 per cent caliche. The greatest growth in this series was found in the cultures approximating an equal mixture of sand and caliche, which is to be

TABLE 3

Average height and dry weight of plants of Larrea *raised in sand, loam, and clay with different percentages of soft caliche, together with the percentages of calcium oxide and magnesium oxide in the ash and their ratios*

	HEIGHT	DRY WEIGHT	CaO	MgO	CaO/MgO
	cm.	*gm.*	*per cent*	*per cent*	
Sand:					
Pure sand	43	14.15	33.62	2.90	11.59
Sand + 10 per cent caliche	56	13.89	39.19	3.48	11.25
Sand + 25 per cent caliche	64	13.70	27.36	2.28	12.00
Sand + 50 per cent caliche	64	16.33	35.05	2.98	11.77
Sand + 75 per cent caliche	49	6.14	32.04	4.38	7.31
Sand + 90 per cent caliche	50	4.49	32.32	5.08	6.36
Loam:					
Pure loam	63	14.37	19.50	3.32	5.87
Loam + 10 per cent caliche	73	13.45	30.25	4.34	6.97
Loam + 25 per cent caliche	57	11.42	31.25	3.90	8.06
Loam + 50 per cent caliche	60	11.98	31.30	5.10	6.14
Loam + 75 per cent caliche	48	5.42	31.70	4.90	6.46
Loam + 90 per cent caliche	54	6.26	25.53	5.00	5.11
Clay:					
Pure clay	69	29.21	31.56	3.86	8.17
Clay + 10 per cent caliche	86	21.76	20.24	2.06	9.82
Clay + 25 per cent caliche	75	18.69	31.30	3.97	7.88
Clay + 50 per cent caliche	67	18.21	28.97	3.91	7.46
Clay + 75 per cent caliche	49	12.76	27.73	3.87	7.16
Clay + 90 per cent caliche	40	7.02	34.30	6.02	5.69
Pure caliche	20	8.00	33.65	5.74	5.86

attributed to an increase in the water-holding capacity of the sand due to the caliche.

In the loam series the greatest growth was found in 90 per cent loam + 10 per cent caliche, while there is evidence of a tendency for growth to be less with increasing percentages of caliche.

In the clay series the greatest growth was again registered in the culture 90 per cent clay + 10 per cent caliche, and a marked decrease was found in the amount of height growth with increasing percentages of caliche.

The figures given in table 3 for dry weight of tops in the several cultures are

more significant of the growth of the plants than are the data on height. The average dry weight per plant was greater in the pure caliche cultures than in any of the cultures with soil plus 90 per cent caliche. The differences are small, however, and of little significance in comparison with the differences exhibited between all dry weights for cultures with high percentages of caliche and those with low percentages. In the sand series the greatest dry weight, as well as the greatest height growth, occurred in the culture 50 per cent sand + 50 per cent caliche. With a further increase in the percentage of caliche there was a sharp falling off in the dry weight. In the loam series the greatest dry weight was found in the plants in pure loam, with a slight decrease accompanying the increased percentages of caliche. In the clay series the dry weight fell rapidly and consistently with increasing caliche. The maximum dry weight per plant was 16.33 gm. in the sand series, 14.37 gm. in the loam series, and 29.21 gm. in the clay series. Considering each series separately and comparing the dry weight in the cultures with soil plus 90 per cent caliche and those in the pure soils, one sees that the high content of caliche reduced the growth to less than one-third in sand, less than one-half in loam, and less than one-fourth in clay.

A complete analysis of the soil conditions responsible for the differences in growth is not possible in connection with this preliminary investigation. No determinations of soluble salts were made, but this condition is being followed in detail in work now in progress. The determinations of pH for the loam series gave readings ranging from 8.05 for the pure loam to 8.60 for the culture with 10 per cent loam + 90 per cent caliche. In the clay series the readings were 8.0 throughout the series. The moisture equivalents of the basic soils are 4.29 for the sand, 9.00 for the loam, 25.21 for the clay, and 25.56 for caliche material under $\frac{1}{80}$ inch diameter. These figures mean that under the uniform but low water supply given to all the plants the comparative growth and dry weight in the three soils are to be attributed to the differences in available water. The differences between the growth in clay on the one hand and loam and sand on the other are greatest in the pure soils and those with low percentages of caliche, and become less as the amounts of caliche are increased and the texture of the soil in the three series is thus rendered more uniform.

The calcium and magnesium contents of the ash of the stems and leaves were determined to show the influence of the different soil mixtures on the absorption of these elements. Samples were prepared from each of the cultures, as raised in duplicate, but only one determination was made for each cultural condition, samples containing equal parts by weight of the pulverized material being used.

The material was prepared for analysis by the methods of the Association of Official Agricultural Chemists (1924 revision, p. 30). For determining the calcium the tentative volumetric method given in section 6, page 41 of that publication was followed and the tentative gravimetric method for magnesium given in section 7, page 42 was adopted up to the point where the sample is ignited before weighing. For the final determination of magnesium the

volumetric method of Handy (1908) as given in Scott (1922, ed., 2, revised) was used. The results of these analyses are presented in table 3 as percentages of the weight of ash used for the determination. The Ca/Mg ratios are also given.

In table 4 are shown the percentages of CaO and MgO and the Ca/Mg ratios found for aerial portions of *Larrea* bushes growing on four characteristic areas of this plant. The same methods were used in obtaining these data, which were obtained in connection with another piece of work not yet published. On two of the areas caliche was very prevalent, whereas on areas III and IV caliche was absent to a depth of 4 feet or more.

Inspection of these data shows that the presence of caliche in the soil does not reduce and may possibly increase the absorption of both calcium and magnesium. The soil type, however, seems to exert a more pronounced influence upon the percentages of calcium and magnesium found in the ash, the sand series yielding the highest values, in general, followed by the loam and clay series respectively. From the results obtained for the pure caliche cultures it

TABLE 4

Percentages of calcium oxide and magnesium oxide, and their ratios, in the ash of Larrea *from two localities with caliche near the surface and two without it*

	AREA	CaO	MgO	CaO/MgO
Caliche near surface	I	22.45	6.12	3.67
	II	16.86	4.77	3.66
Caliche absent	III	15.43	4.80	3.22
	IV	24.90	6.28	3.96

is evident that sufficient calcium and magnesium were available to satisfy completely the needs of the plants. In only three of the "mixed" cultures was the calcium content greater than in the pure caliche culture, and in only one instance was the magnesium content greater.

It is evident that the amounts of calcium and magnesium available to plants in normal desert soils are great enough to give only slight increases, if any, in the amount of these elements in the ash in the case of plants reared in soils with high percentages of poorly available calcium. The conclusion is reached that the physical properties of the soils highly impregnated with calcareous material are more important than the chemical ones in causing the reduction in growth found in the case of *Larrea*.

It is interesting to note that the calcium-magnesium ratios are consistently greater for the plants grown in culture than for those which developed under natural conditions. Since the amounts of magnesium absorbed are nearly equal in the two cases the difference is due chiefly to the greater amounts of calcium taken in by the cultured plants. The latter were much younger and

smaller plants and their roots systems were neither so extensive nor probably so well established. It may be that young *Larrea* shrubs have a higher calcium requirement than older plants. Calcium is thought to be closely related to the formation of hydrophilic colloids within a plant and the hydrophilic colloids are, in turn, intimately concerned in drought resistance. The formation of relatively larger quantities of hydrophilic colloids in young plants would serve as a safeguard against drought until they became thoroughly established.

The calcium content of *Larrea* is relatively high and shows a general agreement with the percentages found by Richardson (6) for certain dune plants from the south shore of Lake Michigan. *Artemesia caudata* contained 35.47 per cent calcium oxide; *Prunus pumila* 44.13 per cent; and *Quercus coccinea tinctoria* 28.86 per cent according to his analyses.

The number of ash analyses which could be included in this investigation was too small to establish definite conclusions in regard to the influence of the various soil conditions studied upon calcium and magnesium absorption. However, the data obtained indicate that the presence of soft or broken caliche probably either increases the calcium content or at best does not lower it; that the physical properties of the soil are more important for the growth of *Larrea* in the desert soils used, than is the chemical constitution, and that the calcium content is relatively high and may have a direct bearing upon the drought resistence of the creosote bush.

SUMMARY

In the southwestern United States, layers of calcareous hardpan, or "caliche," are abundant over extensive areas.

The formation of caliche is due primarily to the interrupted penetration of rain water under arid conditions. Various modifying factors are involved in the formation of caliche in different situations. It occurs in hard layers and in amorphous masses, in both of which forms it has an important influence on the physical and chemical properties of the soil and on the development of the root system.

The maximum water content is very low for the hard layers of caliche (3.2 to 6.5 per cent) but higher for the softer masses (12.9 to 17.3 per cent). Even the thinnest layers of caliche greatly retard the upward or downward movement of water in the soil. Evaporation from caliche which is underlaid by water is less than from an equal surface of a porous cup atmometer.

Considerable differences of moisture content may exist in bodies of soil separated by a layer of caliche. Caliche will convey water more rapidly from soil beneath it to soil above it than it will from soil to the atmosphere.

Roots are unable to penetrate the silicified hard layers of caliche and their distribution is thereby seriously interfered with. The abundance of caliche in the soil of bajadas appears to be an important condition in determining the low and open character of the vegetation, chiefly restricted in such localities to the creosote bush, *Larrea tridentata* (= *Covillea tridentata*).

Cultures of *Larrea* were made in 18 types of soil, varying in texture and percentage of soft caliche. The poorest growth was made in pure caliche; the best growth in soils containing equal amounts of caliche and sand, loam, or clay respectively, or these soils with lower percentages of caliche. The dry weight of the plants decreased with increasing percentages of caliche.

The percentages of CaO in the ash of the cultured plants varied from 20.24 to 39.19 and those of MgO from 2.06 to 6.02. The varying amounts of caliche in the soils were without influence on the amounts of CaO and MgO in the ash. This result has been confirmed by data from field material taken on soils with and without surface caliche.

The fact that the Ca/Mg ratio of the young cultured plants is consistently higher than that of mature plants growing in their natural habitats is due to the higher CaO, and may indicate greater drought resistance on the part of the young plants.

The chemical properties of highly calcareous soils appear to be of less importance than the physical in relation to the growth of *Larrea*.

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PLATE 1

FIG. 1. Caliche from uppermost layer (left). The hard surface 1 cm. thick is underlaid by softer material. Hard layer of caliche 5 cm. thick (right).

FIG. 2. Stone covered with caliche in stalactite form, from roof of small cavity in the soil (left). Recemented type of caliche with many air spaces (right).

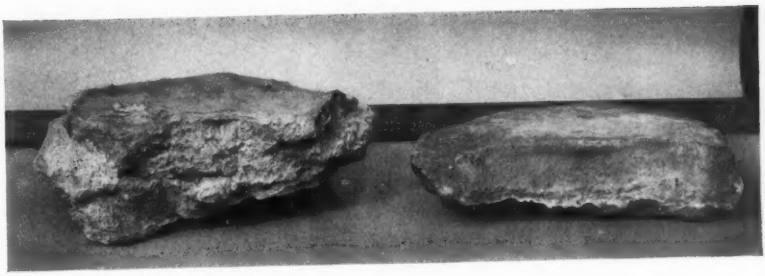

FIG. 1

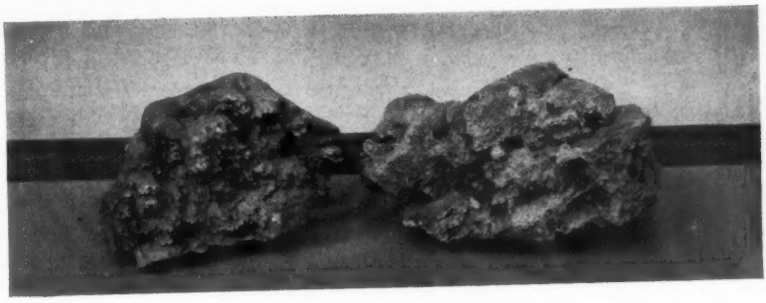

FIG. 2

INFLUENCE OF RYE AND OAT STRAWS UPON THE GROWTH AND YIELD OF CERTAIN VEGETABLES[1]

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Among the soil problems which arise in connection with truck farming the lack of sufficient quantities of organic matter is of great importance. Much work has been done at the Rhode Island Agricultural Experiment Station in connection with the efficiency of substitutes and supplements for stable manure in vegetable rotations. Fertilizer chemicals combined with stable manure have given better yields than stable manure alone. Green manure and fertilizer chemicals have failed to produce as high yields as fertilizer chemicals and stable manure. Combinations of stable manure, green manure, and fertilizer chemicals have proved very efficient (1, 2).

In addition, it was thought that another method of substitution might be the use of straws coupled with ample quantities of fertilizer chemicals. To this end experiments were carried on over a period of years by growing certain vegetables in pots using soils to which varying amounts of straw were applied.

EXPERIMENTAL METHODS

The crops were grown in Wagner pots from seed or small transplants to marketable maturity. Table 1 gives data showing variety, soil source, planting and harvest dates, and number of plants in each pot. There were three pots for each culture. Each pot was filled with carefully screened soil in which the weighed quantities of organic matter were mixed. Two pounds of beach sand was also mixed into the soil for each pot. The soil (Merrimac silt loam) came from two sources. Plat 102 is an agronomy plat which has had chemical fertilizers annually since 1904 for the growing of vegetables. The pH of this soil was 5.2 and the organic matter content accumulated from green manure and crop residues was calculated to be 1.64 per cent. The soil which was used with oat straw was taken from an uncultivated area beside the market garden plats and had a pH of 5.5 and an organic matter content of 4.57 per cent.

The organic matter used was from four sources. Rye straw I, which was, 2 years old and partially decayed, was taken from a station straw stack. Rye straw II came from the base of an old rye stack on a nearby farm and showed little indication of soft decay. Rye straw III was from the same lot as

[1] Contribution No. 427 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

rye straw II but had been allowed to decay a year longer. The oat straw came from a nearby farm and was taken from the base of an old stack which had

TABLE 1

Miscellaneous data connected with growth of crops in Wiley pots

CROP	PLANTED FROM	NUMBER OF PLANTS	SOIL SOURCE	DATES		VARIETY
				Planting	Harvest	
Rye Straw I:						
Lettuce....	Plants	8	Plat 102	June 20	Aug. 9	Cos
Celery.....	Plants	8	Plat 102	June 22	Oct. 22	Golden Plume
Beets.....	Seed	7	Plat 102	June 20	Oct. 29	Early Wonder
Carrots....	Seed	6	Plat 102	May 15	Oct. 31	Danvers Half Long
Onions.....	Plants	12	Plat 102	June 19	Oct. 30	Yellow Globe Danvers
Rye Straw II:						
Lettuce....	Plants	7	Plat 102	May 23	July 16	
Celery.....	Plants	7	Plat 102	June 5	Sept. 19	
Beets.....	Seed	8	Plat 102	May 21	Sept. 24	
Carrots....	Seed	9	Plat 102	May 22	Oct. 2	
Onions.....	Seed	10	Plat 102	May 21	Oct. 3	
Spinach....	Seed	10	Plat 102	May 22	June 30	Savoy Bloomsdale
Rye Straw III:						
Lettuce....	Plants	7	Plat 102	May 29	July 15	
Celery.....	Plants	7	Plat 102	June 19	Oct. 2	
Beets.....	Seed	8	Plat 102	May 29	Sept. 18	
Carrots.....	Plants	10	Plat 102	May 29	Oct. 26	
Onions.....	Plants	10	Plat 102	June 1	Oct. 2	
Spinach....	Seed	10	Plat 102	May 29	July 9	
Oat Straw:						
Lettuce....	Seed	8	Market Garden Roadway	May 17	July 9	
Celery.....	Plants	7	Market Garden Roadway	June 11	Sept. 10	
Beets.....	Seed	7	Market Garden Roadway	May 17	Sept. 20	
Carrots....	Seed	8	Market Garden Roadway	May 17	Oct. 2	
Onions.....	Seed	10	Market Garden Roadway	May 17	Oct. 24	
Spinach....	Seed	10	Market Garden Roadway	May 17	July 1	

been exposed to the weather for some time. This material was black and soft and appeared to be well decayed. Table 2 gives the analyses of these organic

matter materials. Varying amounts of these materials were applied to groups of three pots. These amounts were as follows, in tons per acre:

	LOW	MEDIUM	HIGH
Rye I	4.4	8.8	13.3
Rye II	3.3	13.3	20
Rye III	3.3	13.3	20
Oat	3.3	13.3	20

The fertilizer applications were made in two quantities in all cases with the exception of rye straw I. In that year an attempt was made to determine the optimum fertilization, and three levels were used. Table 3 shows the amounts of the initial fertilization and the totals of such additional applications as were made during the growing periods. At various times during the growing season, soil nitrate determinations were made and in accordance with these results

TABLE 2

Composition of organic materials

ORGANIC MATERIALS	WATER	ORGANIC MATTER	NITROGEN*	P_2O_5*	K_2O*
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Rye Straw I	61.8	20.8	1.37	0.62	0.53
Rye Straw II	65.4	31.7	0.72	0.26	0.54
Rye Straw III	72.2	23.1	1.02	0.28	0.43
Oat Straw	77.3	19.1	2.58	0.90	0.68

* Dry matter basis.

additional applications of nitrate of soda were made when needed. Lime was also supplied at the rate of 2 tons of limestone per acre.

Because it was realized that the moisture content of the soil was likely to be affected by the quantity of organic matter supplied, with rye straw I an attempt was made to evaluate this factor. Table 4 gives the yields with 15, 22, and 30 per cent soil moisture. In so far as was possible these levels were maintained by watering and weighing the pots at the same time each day. The moisture levels were adjusted by adding enough water to bring the pot up to a predetermined weight.

Determinations of moisture on the agronomy field plats have shown that the average range in 1929 and 1930 was between 17.7 and 27.0 per cent (6). In consideration of this fact and of the data given in table 4, it was decided that for the other three organic sources the soil moisture should be made up to 25 per cent, thus somewhat approximating field conditions.

All crops were grown in the summer glass house in pots on small trucks which were wheeled out of doors on dry days. After harvest the roots were sepa-

rated from the tops and weighed. Then both portions were dried and the air-dry weight taken. Selected samples were ground and stored for analysis.

DISCUSSION OF EXPERIMENTAL RESULTS

In similar experiments a usual procedure is to attempt to control all of the known factors with the exception of one which is left free to operate uncontrolled. If it is not possible to achieve control of a factor an attempt to meas-

TABLE 3

Applications of fertilizer in grams per pot

Fertilizer applied at planting time

	REGULAR				HIGH			
	Rye I*	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat
Leuna saltpetre	0.7	0.7	0.7	0.7	1.0	1.0	1.0	1.0
Hoof meal	4.0	4.0	4.0	4.0	13.7	4.0	4.0	4.0
Superphosphate	3.5	3.5	3.5	3.5	5.25	5.0	5.0	5.0
Thomas slag	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Sulfate of potash	2.2	2.5	2.5	2.5	2.8	3.0	3.0	3.0

Additional fertilization during growth of crops

	RYE I			RYE II		RYE III			OAT	
						$NaNO_3$				
	$NaNO_3$	K_2SO_4	Superphos	$NaNO_3$	K_2SO_4	O and low O.M.†	Medium and high O.M.	K_2SO_4	$NaNO_3$	K_2SO_4
*Lettuce	0.5	0.5	2.0	3.0	....	2.0	4.0	....	0.5	0.5
Celery	1.5	0.5	2.0	6.0	2.0	8.0	10.0	1.0	3.0	1.0
Beets	2.0	0.5	2.0	7.0	2.0	8.0	11.0	1.0	4.5	2.0
Carrots	1.5	0.5	2.0	4.0	1.0	6.0	8.0	1.0	3.5	1.5
Onions	1.5	0.5	2.0	3.0	....	3.0	6.0	1.0	2.5	0.5
Spinach	....	....	....	1.0	....	3.0	3.0	....	0.5	0.5

*With rye straw I a medium fertilizer level with the medium organic matter level was used in addition to the regular and high amounts. This consisted of 1.0 gm. Leuna saltpetre, 9.0 gm. hoof meal, 4.55 gm. superphosphate, 3.5 gm. Thomas slag, and 2.8 gm. sulfate of potash.

† Organic matter.

ure the results of its change is often made. In this case the moisture, fertilizer, and organic matter factors were controlled. The results in table 4 indicated that making up the moisture daily to 25 per cent would give an average moisture of approximately 22 per cent and that optimum crops might be expected with this moisture.

For the fertilizer a medium or regular application was chosen (table 3), but in order to determine to what extent the yields were optimum with this fer-

tilizer level a higher quantity was used in all cases with all organic sources except rye I. With such control of these two factors and with environmental factors the same for all the crops in the experiment it was thought that the effects of applications of different quantities of organic matter might be expected to show up in the growth of the vegetable crops.

In table 5 are given the dry weight yields of the various crops as grown with different quantities of the four organic sources. In general a definite increase in yield was noted with the increasing quantities of rye I and oat straw. This did not hold true with rye II and rye III. In the latter years some other factor seemed to be interfering with any effect which the higher organic matter might

TABLE 4

Dry weight yields, in grams, of crops grown with varying moisture and organic levels

Rye I

ORGANIC MATTER APPLIED	LETTUCE			CELERY			BEETS			ONIONS			CARROTS			SPINACH*	
	Per cent moisture																
	15	22	30	15	22	30	15	22	30	15	22	30	15	22	30	25	30
Regular fertilizer																	
No organic matter	..	47	...	..	39	...	...	103	...	..	51	...	...	170	...	51	56
Low organic matter	38	...	70	38	...	115	99	...	142	50	...	93	90	...	201	58	54
Medium organic matter	40	51	63	52	129	123	100	121	96	45	57	106	105	193	247	69	69
High organic matter	48	67	60	53	124	135	94	107	114	47	73	151	87	181	201	....	....
High fertilizer																	
No organic matter	..	...	...	..	...	...	...	...	...	..	...	...	...	...	...	55	....
Low organic matter	33	...	43	44	...	170	99	...	187	51	...	157	111	...	303	61	....
Medium† organic matter	49	52	41	49	146	176	95	189	191	48	103	170	89	257	288	73	72
High organic matter	51	69	64	61	129	158	104	131	129	67	105	148	105	230	254	....	....

* The organic matter source with spinach was oat straw.

† Medium fertilizer (see table 3).

have had. In order to investigate the cause of this effect, nitrate determinations were made on the soil in the pots from time to time. These results are given in table 6. In all cases the nitrate nitrogen content was much lower where the high quantity of organic matter had been added.

The depressing effect upon soil nitrates of undecomposed straw has been noted by Murray (4), Scott (5), and others. Waksman (7) and his coworkers have shown that nitrate accumulation is related directly to the carbon and nitrogen content of the organic matter applied to the soil.

If the nitrogen content is more than 2 or 2.5 per cent, no nitrogen starvation can set in, but ammonia will be liberated. If the nitrogen content of the organic matter is about 2 or 2.5 per cent, a temporary nitrogen starvation may set in lasting a few days followed by liberation of

TABLE 5

Dry weight yields in grams, of crops grown with varying levels of oat and rye straw

ORGANIC MATTER APPLIED	LETTUCE				CELERY				BEETS				CARROTS				ONIONS				SPINACH			
	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat
Regular fertilizer																								
No organic matter	47	120	81	133	39	236	245	250	103	309	324	352	170	397	390	399	51	275	221	250	....	52	56	51
Low organic matter	....	116	86	138	....	216	225	254	....	271	318	360	....	384	365	420	....	275	255	315	....	50	54	58
Medium organic matter	51	75	87	160	129	181	224	269	121	234	348	380	193	280	408	488	57	219	208	284	....	23	47	69
High organic matter	67	54	80	171	124	155	231	302	107	233	332	427	181	236	443	520	73	154	201	365	....	14	44	73
High fertilizer																								
No organic matter	....	122	85	135	....	246	243	253	....	351	332	371	....	424	382	414	....	223	219	261	....	53	60	55
Low organic matter	....	117	91	135	....	230	246	253	....	323	309	371	....	412	348	436	....	242	214	281	....	47	59	61

ammonia. If the nitrogen content of the organic matter is about 1 per cent or less, a lasting nitrogen starvation may set in which may be corrected by the addition of inorganic nitrogen fertilizers.

Table 2 shows that the nitrogen content of rye II and rye III closely approached 1 per cent or less and this fact connected with the soil nitrate results (table 6) may explain the lower yields with high organic matter in the cases of rye II and rye III. In other words, the decomposing straw kept the soil nitrates so low that the growth of the plant was affected and constituted the

TABLE 6

Nitrate nitrogen, in parts per million, in soil of organic matter pots

DATE OF SAMPLING	BEETS (RYE II)		BEETS (RYE III)		CARROTS (RYE III)		SPINACH (RYE III)	
	No O.M.*	High O.M.	No O.M.	High O.M.	No O.M.	High O.M.	No O.M.	High O.M.
August 1	188	2	.....	.....	.....	.....	.....	.....
September 4	12	2	.....	.....	.....	.....	.....	.....
June 15	.....	.....	62	28	.....	.....	.....	.....
June 23	.....	.....	.....	.....	.....	.....	54	22
July 9	.....	.....	12	8	43	3	.....	.....

* Organic matter.

TABLE 7

Total nitrogen content, in milligrams per 100 gm. of dry tissue, of crops grown without organic matter and with high organic matter applications

CROP	OATS		RYE II		RYE III	
	No O.M.*	High O.M.	No O.M.	High O.M.	No O.M.	High O.M.
Beets	4,184	6,063	2,119	1,695	5,220	5,549
Carrots	3,425	4,879	1,740	1,019	4,072	4,612
Celery	3,174	4,749	3,184	2,134	4,086	4,223
Lettuce	2,348	3,234	2,840	1,146	2,166	2,198
Onions	3,079	5,338	3,558	1,967	3,608	3,904
Spinach	.....	.....	1,649	328	2,257	1,521

* Organic matter.

unknown limiting factor which masked any effect on yield that increasing quantities of organic matter might have produced. Further evidence that the supply of available nitrogen may have been the limiting factor is given in table 7.[2] It will be noted that with the highest quantity of oat straw the total crop nitrogen was consistently greater with all the crops, whereas in the case of rye II the opposite results are found. With rye III the large applications of nitrogen of that year balanced the detrimental effects of the organic matter with

[2] The chemical analyses were made by Mr. J. B. Smith and his co-workers.

lettuce, beets, and carrots (table 5). The plants which received the high application of organic matter had some reserve nitrogen, as shown by table 6. The results with the other crops—onions, celery, and spinach—are not so conclusive.

With rye I and oat straw the total nitrogen content of the straws was perhaps sufficient to supply the requisite nitrogen for decay without interfering with the growth of the plants.

Some writers have doubted that extreme cases of nitrogen starvation brought about by resistant organic matter in the soil, could be corrected by applications of nitrogen fertilizers. Hill (3), using pure cellulose, got little effect with an 8-ton application of sodium nitrate with corn. In this experiment the nitrate nitrogen deficiency with rye II and III having been with additional applications of sodium nitrate were made (table 3). This was tried particularly with rye III. It will be noted, however, that even with 3,030 pounds of nitrate of soda per acre with beets the yield from high organic matter was barely held up to the no-organic matter yield. Thus in this experiment, although benefit was derived from the use of additional nitrogen, it is doubtful whether the unruly nitrogen starvation factor could have been entirely eliminated by additional fertilizer applications.

SUMMARY

Further evidence is given to show the inhibitory effect upon plant growth of large quantities of poorly decayed organic matter. By the use of various levels of rye and oat straws of different composition and stages of decomposition an attempt was made to determine the value of these sources of organic matter in connection with the growth of lettuce, celery, beets, carrots, onions, and spinach. These pot tests seem to show the following:

Such resistant organic matter sources have doubtful value as substitutes for stable manure. This is especially true where the nitrogen content of the straws is very low.

Evidence is given to show that, although it may be possible to balance the inhibitory effect which accompanies the decomposition of low nitrogen straws by very large applications of nitrogen, these are of necessity so large as to be out of reason for field practice.

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A STATISTICAL STUDY OF NITROGEN FIXATION BY CLOVER PLANTS[1]

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In 1869, Galton applied to the problem of biological inheritance the mathematical laws of probability, the practical application of which had hitherto been of use only in life insurance and gaming; thus were laid the foundations of the science of biometry. Since that time under the leadership of Galton, Pearson, Yule, Fisher, and others this branch of statistics has been developed into a powerful tool which is becoming more and more indispensable to the research worker in the biological sciences. At present the progress of biometry suffers from two handicaps, viz., lack of use and misuse. All too few biologists avail themselves of the many valuable methods at their disposal for analysis of data obtained in the laboratory or field; until this is done, the statistical methods developed will suffer from an academic formality and idealization which restricts their application to actual data. The success of the Rothamsted Experimental Station in overcoming this handicap lends point to the argument. On the other hand, the misapplication of statistical formulas to observations is primarily due to the carelessness of the user in failing to ascertain, or to understand, the principal assumptions involved in the derivation of the formulas. This unfortunate circumstance will be largely eliminated when the research student acquires a proper acquaintance with statistical methods and thus disarms those critics who carp at the application of anything resembling mathematics to the so-called "descriptive sciences."

As an example of the value of statistical analyses of data this study of the nitrogen fixation process in red clover is presented. In the experiments to be described, data were gathered from 200-odd plants. Because of the large number and the variability in the measurements, little information could be gained by a study of the data without a statistical analysis. However, when subjected to such an analysis, quite definite conclusions were apparent. The primary purpose in the experiments was to obtain some information in regard to the distribution of several variables characteristic of the fixation process. This knowledge was desired in order to apply the proper statistical treatment to data obtained in future experiments made under conditions similar to the ones described. Naturally the specific conclusions reached are more or less restricted to experiments thus circumscribed, but the general nature of the results may be of interest to many workers engaged with the problem of nitrogen fixation in the leguminous species. Similar studies under different conditions, e.g., in the field, or with different species, are desirable not only for their statistical value but because they should elucidate some of the interrelationships that obtain in the fixation process. In addition to the practical benefits, the methods of attack and the nature of the questions answered by an analysis of this kind might interest other research workers irrespective of their concern with the specific problem studied.

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EXPERIMENTAL

Information was desired on the statistical characteristics of two populations, viz., clover plants grown in the open in the greenhouse and plants grown in more easily controlled culture solutions. For this reason the experiment was divided into two parts according to substrate on which the plants were grown: (*a*) nitrogen-poor sand plus modified Crone's solution, (*b*) nitrogen-free Crone's agar. In both parts aqueous suspensions of *Rh. trifolii*, strain 209, prepared from a 48-hour agar slant, were used for inoculation. The technique used in setting up the experiments was that described by Hopkins, et al. (5).

Growth of plants in agar

Plants were grown in 800-cc. glass bottles, each containing 250 cc. of Crone's agar medium adjusted to pH 7.0 by the addition of about 10 gm. calcium carbonate. Six germinating clover seeds were placed in each bottle in a circle, care being taken that the distance between them was fairly uniform. In this way 60 bottles were prepared, of which 40 were inoculated with *Rh. trifolii;* the other 20 were kept uninoculated as controls. Litmus milk was used to test the sterility of the seed and the purity of the inoculum.

At the time of harvest, nodules and leaves were counted, the length of the roots and tops was measured, and then the plants were placed in 20-cm. test tubes with 2 cc. conc. H_2SO_4 and kept until they could be analyzed for total nitrogen.

Growth of plants in sand

Plants were grown in 1-gallon earthenware jars each containing 2,500 gm. of nitrogen-poor sand with which 5 gm. of sterilized $CaCO_3$ was well mixed. Each jar was provided with a glass tube about 2.5 cm. in diameter, leading to within 3 cm. of the bottom and extending about 10 cm. above the top of the jar. This tube was closed at the top with cotton and through it sterile water or modified Crone's solution was added to the sand.

After sterilization at 15 pounds pressure for 8 hours, each jar was seeded with 10 clover seeds under aseptic conditions. Thirty such jars were inoculated with *Rh. trifolii*, and fifteen were left uninoculated as controls. The surface of the sand was then covered with a 5-mm. layer of sterile ground cork to reduce contamination from the air. Through the tube 200 cc. of modified Crone's solution and 200 cc. of sterile distilled water were added. All jars were watered daily with the same quantity of water, the quantity being varied as the weather required.

Nitrogen determination

Total nitrogen was determined by a micro-method similar to those described by Chiles (1) and Walters (9). Each plant was digested with the usual Kjeldahl reagents, and the digest nesslerized directly. As suggested by Looney (6), gum

ghatti solution prepared according to the directions of Folin (3) was used as a protective colloid in the nesslerization.

This micro-nitrogen determination was checked against the regular macro-determination generally used in this laboratory, as follows: Sets of 200 and 300 clover seeds, accurately counted, were digested and analyzed by the macro-Kjeldahl method. The results obtained were used to calculate the average nitrogen content per seed. By the micro-method already described, seeds were analyzed in lots of two, three, or five, and the results compared with those obtained by the macro-methods. The average nitrogen content by the micro-method was 0.0975 mgm. per seed; that by the macro-method, 0.0985 mgm.

The reliability of the method was further examined by a series of recovery tests. For each series five seeds were digested, the digestion mixture was diluted to 100 cc., 20 cc. of which was nesslerized directly. To a second aliquot of 20 cc., 0.05 mgm. of N in the form of $(NH_4)_2SO_4$ was added; this mixture was then boiled, digested, and, after cooling, nesslerized. To a third aliquot of 20 cc. of the same digested sample, 0.10 mgm. $(NH_4)_2SO_4$-N was added, and the total nitrogen was determined after boiling and digestion. The latter is, in fact, not a true recovery test, because the quantity of nitrogen to be recovered is already in the form for direct nesslerization without previous digestion. The procedure was believed, however, to be a fair check on the micro-method. The recovery of added nitrogen ranged from 94.0 to 104.0 per cent. Though the error appears fairly large, it is, in consideration of the small quantities to be recovered, small. Variations in nitrogen content of the plant were so great, that an error of +4 or −6 per cent would be of little consequence to the final results. Because of the close checks with the macro-Kjeldahl method and because of the fair recovery tests, this micro-nitrogen method was adopted for the work.

DISCUSSION OF RESULTS

The plants grown in agar were placed in the greenhouse February 24, 1931, and harvested 94 days later. At the time of harvest, the plants were tall and inclined to be spindly and pale green; the leaves were numerous but small. The plants grown in sand were seeded March 4, 1931, and harvested after 71 days. These plants were tall, sturdy, dark green, and had large leaves. Summaries of the observations and measurements are given in tables 1 (agar series) and 2 (sand series). The nitrogen content of the uninoculated controls in the agar series varied from 0.16 to 0.30 mgm. This entire range was just equal to that of each *class* of the inoculated plants; therefore, the mean value of nitrogen in the controls was used to determine the *nitrogen fixed* in this series. The same was true of the sand series except that the controls ranged from 0.30 to 1.00 mgm.; this difference in the nitrogen content of the controls lies in the small quantity of nitrate nitrogen available to the plants grown in sand. The number of nodules, length of stem, and length of roots were divided into classes whose limits were so chosen that no actual measurement could fall on a class

limit; e.g., the class limits of the length of stem and roots were of the type 5.25 to 6.25 cm., but the measurements were made only to the nearest 0.5 cm. The data were grouped in all cases so that 10 to 12 classes would be formed of the 200-odd observations; this required that the class range should be somewhat more than one-quarter of the standard deviation as advised by Fisher (2, p. 50). However, in order to obtain a sufficient number of classes (12, p. 79), the grouping adopted was necessary; the error in the estimates of the parameters calculated from the data so grouped is about 2 to 3 per cent at the

TABLE 1

Inoculated clover plants grown in agar

NITROGEN FIXED		NODULES		LENGTH OF STEM		LENGTH OF ROOT		LENGTH OF ROOT / LENGTH OF STEM		LEAVES	
Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Number	Frequency
mgm.	*no.*	*no.*	*no.*	*cm.*	*no.*	*cm.*	*no.*	*no.*	*no.*	*no.*	*no.*
<0.30	3	<7.5	21	<4.25	5	<9.25	11	0.0 –1.01	14	4	3
0.30–0.45	5	7.5–12.5	49	4.25– 5.25	3	9.25–11.25	18	1.01–1.21	22	5	4
0.45–0.60	9	12.5–17.5	45	5.25– 6.25	2	11.25–13.25	22	1.21–1.41	15	6	5
0.60–0.75	22	17.5–22.5	37	6.25– 7.25	10	13.25–15.25	25	1.41–1.61	30	7	20
0.75–0.90	29	22.5–27.5	15	7.25– 8.25	20	15.25–17.25	28	1.61–1.81	25	8	22
0.90–1.05	25	27.5–32.5	12	8.25– 9.25	21	17.25–19.25	30	1.81–2.01	20	9	38
1.05–1.20	23	32.5–37.5	5	9.25–10.25	47	19.25–21.25	13	2.01–2.21	22	10	58
1.20–1.35	20	37.5–42.5	6	10.25–11.25	33	21.25–23.25	12	2.21–2.41	17	11	38
1.35–1.50	17	>42.5	6	11.25–12.25	27	23.25–25.25	14	2.41–2.61	10	12	6
1.50–1.65	16			12.25–13.25	18	25.25–27.25	5	2.61–2.81	8	13	3
1.65–1.80	11			13.25–14.25	7	27.25–29.25	8	2.81–3.01	4	>13	2
1.80–1.95	7			>14.25	3	29.25–31.25	6	3.01–3.21	2		
1.95–2.10	4					>31.25	4	>3.21	7		
>2.10	5										
Total....	196										

maximum. The ratio $\frac{\text{length of root}}{\text{length of stem}}$ was calculated from the data as a measure of the stockiness of the plant.

From tables 1 and 2, it is seen that the outstanding differences between the measurements of the plants grown in agar and those grown in sand are in the amount of nitrogen fixed and in the number of nodules. While inoculated plants grown in agar fixed up to 2.1 + mgm. of nitrogen per plant, inoculated plants grown in sand fixed more than 13.0 mgm. This difference is due in part to much better opportunity for growth afforded to the plants grown in sand. The plants on agar in bottles are restricted by the poor gas exchange because of the cotton plug; carbon dioxide becomes a limiting factor in the growth and in the fixation of nitrogen (10). Possibly the number of nodules on

the plants grown in sand is greater because the root nodule organism may be able to move about more freely in the loose sand than in the compact agar medium. On agar it can penetrate the surface only at those places where the plants penetrate it. From this it might be presumed that on account of the dependency of the bacteria upon the plant to get below the agar surface, the bacteria will invade those root hairs of the plant that are near the crown and along the tap root. In sand, however, the bacteria are free to move independently of the plant. The invasion, therefore, is not necessarily restricted to the

TABLE 2

Inoculated clover plants grown in sand

NITROGEN FIXED		NODULES		LENGTH OF STEM		LENGTH OF ROOT		LENGTH OF ROOT / LENGTH OF STEM		LEAVES	
Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Number	Frequency
mgm.	*no.*	*no.*	*no.*	*cm.*	*no.*	*cm.*	*no.*	*no.*	*no.*	*no.*	*no.*
0.0– 1.0	10	<7.5	15	<5.25	9	<11.25	8	<1.01	10	3	2
1.0– 2.0	18	7.5–12.5	15	5.25– 6.25	4	11.25–12.25	3	1.01–1.21	16	4	15
2.0– 3.0	22	12.5–17.5	25	6.25– 7.25	4	12.25–13.25	7	1.21–1.41	29	5	36
3.0– 4.0	25	17.5–22.5	47	7.25– 8.25	20	13.25–14.25	15	1.41–1.61	43	6	41
4.0– 5.0	21	22.5–27.5	14	8.25– 9.25	18	14.25–15.25	26	1.61–1.81	39	7	32
5.0– 6.0	24	27.5–32.5	22	9.25–10.25	29	15.25–16.25	27	1.81–2.01	24	8	27
6.0– 7.0	18	32.5–37.5	6	10.25–11.25	27	16.25–17.25	18	2.01–2.21	12	9	14
7.0– 8.0	17	37.5–42.5	19	11.25–12.25	29	17.25–18.25	21	2.21–2.41	9	10	16
8.0– 9.0	14	42.5–47.5	5	12.25–13.25	20	18.25–19.25	27	2.41–2.61	7	11	5
9.0–10.0	12	47.5–52.5	21	13.25–14.25	19	19.25–20.25	20	2.61–2.81	5	12	1
10.0–11.0	5	.....	...	14.25–15.25	11	20.25–21.25	11	2.81–3.01	0	13	4
11.0–12.0	8	.....	...	15.25–16.25	4	21.25–22.25	5	>3.01	8	14	9
12.0–13.0	6	72.5–77.5	13	16.25–17.25	4	22.25–23.25	4				
>13.0	2	.....	...	17.25–18.25	4	23.25–24.25	5				
						24.25–25.25	0				
						25.25–26.25	5				
Total...	202										

crown and to the tap root, but can take place also in the root hairs along the side roots. The difference in pCO_2 in the atmosphere available to plants is also another factor in the location of the nodules, as will be demonstrated in publication (10). This difference in the location of the nodules will be treated in greater detail under *correlation* in the next section.

STATISTICAL TREATMENT

The methods used to analyze the data given in tables 1 and 2 are discussed in detail in standard texts on statistics, e.g., Fisher (2), Rietz (8) and Yule (12).[2]

[2] Reference to the texts are made by page number whenever necessary but the formulas used are included in our discussion in order to avoid discontinuities in the treatment. The derivation and discussion of these formulas can be obtained from the references given.

Homogeneity of data

In spite of all efforts to maintain uniform conditions throughout the greenhouse, it is possible that the plants in a given series were not grown under identical conditions; this would lead to a non-homogeneous sample. In order to determine whether or not the data are homogeneous, they were tested by the method described by Fisher (2, p. 190). All of the plants grown in one bottle or jar may be considered to be subjected to the same treatment, but plants from different bottles or jars are regarded as being samples from populations with different treatments. Hence the plants in a given bottle may be looked upon as forming a *class* and the test consists essentially in the determination of the significance of correlation *within classes*. This is a type problem in the analysis of variance, the treatment of which is briefly summarized. The two following statistics are calculated from the data:

$$S_E = \sum_{1}^{kn} (x - \bar{x}_P)^2 = n\,(k-1)\,B$$

$$S_T = \mathrm{k} \sum_{1}^{n} (\bar{x}_P - \bar{x})^2 = (n-1)\,(kA + B)$$

x = individual observation (milligrams nitrogen fixed by a given plant)
$\bar{x}_P$ = mean of a class (plants in one bottle or jar)
$\bar{x}$ = mean of all observations
n = number of classes (number of bottles or jars)
k = number of observations in each class

From these two formulas, A and B may be determined, from which the *intra-class correlation coefficient* R can be estimated:

$$R = \frac{A}{A + B}$$

A may be regarded as the variance due to "causes" which observations in one class have in common, and B is the remainder of the total variance of the population. Unless R is significantly different from zero, the sample may be regarded as homogeneous. To test this the statistic z is calculated:

$$Z = 1/2\, ln \frac{V_T}{V_E} \tag{11}$$

$$V_E = \text{variance due to errors} = \sum_{1}^{kn} (x - \bar{x}_P)^2 \div n\,(k-1)$$

$$V_T = \text{variance due to treatment} = k \sum_{1}^{n} (\bar{x}_P - \bar{x})^2 \div (n-1)$$

The distribution of z has been determined for different values of n and k and tables are available which are entered with the value of z, n, and k given by the

data, and when so entered give the probability that the observed value of R is due to chance. Unless the value of this probability is less than 0.05, the observed correlation cannot be held to be significant, and the population sampled is homogeneous. The results of the tests on the homogeneity of the samples from the *milligram nitrogen* and *number of nodules* "populations" are given in table 3. Since some plants were lost in the nitrogen analyses, it was necessary, in order to have the same number for each "treatment," to use only those jars or bottles which furnished a certain minimum number of plants for nitrogen analysis. In the agar series this was taken to be 5, i.e., k equals 5; in

TABLE 3

Homogeneity test on samples of population

	DEGREES OF FREEDOM	SUM OF SQUARES (S)	MEAN SQUARE (V)	INTRA-CLASS CORRELATION (R)	Z	PROB.*
Agar series—nitrogen fixed						
Within classes	116†	17.3497§	0.1496**	0.046	0.1085	>0.05
Between classes	28‡	5.2055‖	0.1859††			
Agar series—number of nodules						
Within classes	148	13,549.0	91.55	0.048	0.1117	>0.05
Between classes	36	4,120.4	114.5			
Sand series—nitrogen fixed						
Within classes	98	978.24	9.982	0.105	0.3300	0.04
Between classes	13	251.00	19.307			
Sand series—number of nodules						
Within classes	189	54,005.9	285.7	0.216	0.4523	<0.01
Between classes	26	18,376.7	706.8			

* Probability that intraclass correlation coefficient is not different from zero, i.e., population is homogeneous.

† $= n(k-1)$; ‡ $= n-1$; § $= S_E$; ‖ $= S_T$; ** $= V_E$; †† $= V_T$.

the sand series, k equals 8. For example, in the sand series all samples from a jar that had less than 8 plants analyzed were eliminated from the test; in case more than 8 plants from a jar were analyzed, the extra samples were eliminated by chance (drawing of a card). A like system was followed in the agar series k equal to 5. Only in the case of the sand cultures did this selection eliminate a large number of samples, but even here the selected population was representative of the original population. The mean of 202 samples was 5.56 $\pm$0.22 mgm. before selection as compared with the 5.93 $\pm$0.32 (112 samples) after selection. The values of the probabilities given in table 3 indicate that only in the case of the number of nodules in sand cultures are there indications

that the populations sampled are not homogeneous. This population will be discussed later when the type of distribution is considered. There is some evidence of a slight intraclass correlation in the population of nitrogen fixed in sand cultures, but the significance of this is doubtful.

Type of distribution

The distribution of the samples of nitrogen fixed and of the number of nodules in both the agar and sand series were compared with the theoretical Gaussian (normal) curves by calculation of the first four moments from the observations. Estimates of the moments were made by the use of the usual formula (2, p. 72):

$$\mu_i = \frac{1}{n} \sum (x - \bar{x})^i$$

n = number of observations
x = individual observation
$\bar{x}$ = mean of n observations
i = 1, 2, 3 ————————

The crude moments, ν_i, were calculated from the grouped data and μ_i determined by applying the usual Sheppard's correction. The values of μ_i so estimated were used to calculate two statistics suitable for measuring departure from the normal curve, viz., α_3 and α_4 (γ_1 and γ_2 in Fisher's terminology).

$$\alpha_3 = \frac{\mu_3}{\mu_2^{3/2}} \qquad \alpha_4 = \frac{\mu_4}{\mu_2^2}$$

Since for the normal frequency curve $\alpha_3 = 0$ and $\alpha_4 = 3$, it is necessary only to determine whether or not the α's calculated from the data are significantly different from the theoretical values. This is done by comparing the difference between the observed value of α_i and its theoretical value with its standard deviation. Unless the observed difference is 3 to 4 times its standard deviation, the departure from normality cannot be held to be significant. For a normal distribution, the standard deviation of α_3 is $\sqrt{\frac{6}{n}}$ and that of α_4 is $\sqrt{\frac{24}{n}}$.

The statistics used to test the normality of the observed distribution of frequencies are given in table 4. The values obtained indicate that the distribution of nitrogen fixed by clover plants in agar or in sand is essentially normal. There is some evidence that α_3, which measures the asymmetry of the curve, is different from its theoretical value, zero, but the deviation is not marked either in sand or in agar. In both sand and agar the value of α_4, which measures the flatness of the curve, is not significantly different from its theoretical value of 3.0. On the other hand, the distribution curve of the number of nodules in both the sand and agar series is quite asymmetric toward the lower values, as indicated by the high value of α_3 for these populations. Since the differences from zero are about 6 times the corresponding standard deviations, the observed asymmetry is certainly significant. In the case of the plants grown in

TABLE 4

Statistical constants on distribution

Distribution	MOMENTS μ_1	μ_2	μ_3	μ_4	α_2	$\frac{\alpha_2 - 0.0}{\text{S.D. } \alpha_2}$	α_4	$\frac{\alpha_4 - 3.0}{\text{S.D. } \alpha_4}$
Nitrogen fixed in agar	0	8.753	10.966	209.49	0.424 ±0.175	2.42	2.734 ±0.348	0.75
Nitrogen fixed in sand	0	10.141	13.920	240.19	0.431 ±0.172	2.50	2.336 ±0.345	1.93
Nodules in agar	0	3.666	7.668	50.803	1.092 ±0.175	6.23	3.778 ±0.348	2.24
Nodules in sand	0	12.497	46.573	572.63	1.054 ±0.172	6.13	3.666 ±0.345	1.93
Nodules in sand (selected)*	0	7.022	8.558	107.80	0.460 ±0.178	2.58	2.186 ±0.356	2.29

* After elimination of those above 50 nodules.

TABLE 5

"Goodness of fit" of theoretical distribution with observed

POPULATION \ DISTRIBUTION	$\varphi(t)$			$\varphi(t) - \frac{\alpha_3}{3!}\varphi^3(t)$			$\varphi(t) - \frac{\alpha_3}{3!}\varphi^3(t) + \frac{\alpha_4 - 3}{4!}\varphi^4(t)$		
	N	X^2	Probability*	*N*	X^2	Probability	*N*	X^2	Probability
I. *Agar series:*									
Nitrogen fixation	12	11.71	0.47	11	5.00	0.92	10	4.46	0.90
Nitrogen fixation (grouping)	2	7.05	0.03	1	4.72	0.03	2	4.32	0.10
Number of nodules	7	49.09	<0.01	6	8.71	0.20	5	8.33	0.15
Number of nodules (grouping)	2	22.12	<0.01	1	6.20	0.01	2	7.37	0.03
II. *Sand series:*									
Nitrogen fixation	12	21.80	0.04	11	13.06	0.30	10	12.71	0.30
Nitrogen fixation (grouping)	4	17.62	<0.01	4	11.53	0.02	3	9.75	0.02
Number of nodules†	7	33.21	<0.01	6	22.10	<0.01	5	20.75	<0.01

* Probability that worse fit would be obtained through random sampling.

† All 13 samples containing more than 50 nodules eliminated.

the sand, part of the asymmetry is due to a second mode appearing in the distribution at about 75 nodules (see table 2). When these samples are eliminated, the value of α_3 falls to a relatively insignificant figure. In neither sand nor agar series is there any intimation of a departure from normality in regard to the flatness of the curve of distribution of nodules as measured by α_4.

The actual distributions found were compared with the theoretical normal curves and with the normal curves partially corrected for the observed asymmetry or flatness by calculating theoretical distributions from various numbers of terms in a Charlier's series (8, p. 114)

$$F(t) = \varphi(t) - \frac{\alpha_3}{3!}\varphi^3(t) + \frac{\alpha_4 - 3}{4!}\varphi^4(t) + \ldots\ldots$$

The normal curve was used as the generating function in this series, i.e., $\varphi(t)$, and by taking one, two, and three terms, the observed distributions were compared with: the theoretical normal curve, the normal curve partially corrected for evidences of asymmetry, and the normal curve partially corrected for asymmetry and flatness. The comparison was made by the χ^2 "goodness of fit" test (2, p. 75). The results of the comparison are given in table 5 and figures 1 and 2. A consideration of these figures and of table 5 is most instructive for our inquiry into the nature of the distributions of the populations. Table 5 shows that the distribution of nitrogen fixed in agar corresponds to the normal curve specified by the first two moments of the data as closely as could be expected from a sample of 200 individuals; in this case the inclusion of the correction terms merely leads to a higher probability that the observed deviations arose from chance. In the sand series, the fit of the observed data on *milligrams nitrogen fixed* to their theoretical normal curve is none too exact, as the probability that a worse fit could be obtained through sampling is only 0.04. However, the addition of the first term that corrects for asymmetry $\left[\frac{\alpha_3}{3!}\varphi^3(t)\right]$ in the series results in a satisfactory agreement between the theoretical and observed values. The distribution of the *number of nodules* in agar is characterized by an asymmetry toward the lower values, as was discussed in connection with table 5. Hence, the uncorrected normal distribution curve does not fit the data, but two terms of the proper Charlier's series describe a curve that shows fair agreement with the observations. On the other hand, the observed distribution of nodules on plants in sand does not agree with any of the three theoretical distributions calculated from the proper series. Since the selected values of these nodules were used for the test, this result is rather surprising in light of the values of α_3 and α_4 for this distribution (see table 5). A glance at the plotted frequencies (fig. 2) shows that the lack of fit is due to alternately high and low frequencies. This suggests that the original counts were not accurately determined, which resulted in an excess in the frequency of one class and a deficiency in the neighboring one. Consultation of the original data verified this hypothesis. Because of the large number and the

small size of many of the nodules in the sand series, an accurate count of the higher values is not obtained in ordinary laboratory practice. As a result of the difficulties in counting the nodules, many of the values are rounded off to the nearest 5 or even 10 units, a practice that probably accounts for the seeming irregularity in the plotted frequencies. In a much larger sample, this inaccuracy in the estimation of the nodules would be greatly eliminated by compensation; however, in the small sample taken in this experiment, it might easily lead to the observed alternate excess and deficiency in neighboring classes.

Further consideration of figures 1 and 2 leads to the suspicion that the relatively high values obtained for the probabilities that the observed frequencies

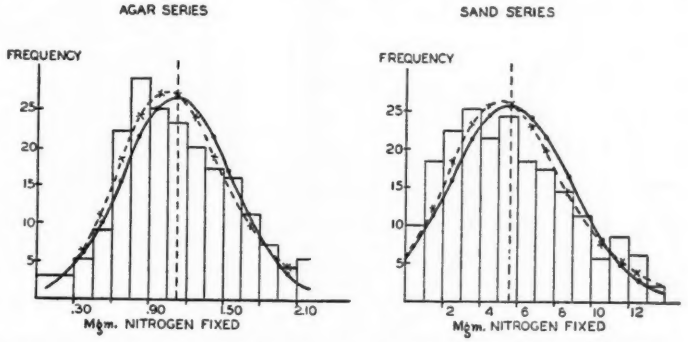

FIG. 1. COMPARISON OF THE OBSERVED DISTRIBUTION OF MILLIGRAM NITROGEN FIXED BY CLOVER WITH TWO THEORETICAL CHARLIER'S SERIES

$\varphi(t)$ is the normal curve of errors

●——● $F(t) = \phi(t)$

x---x $F(t) = \phi(t) - \frac{\alpha_3}{3!}\phi^3(t)$

$$\phi(t) = \frac{1}{\sqrt{2\pi}} e^{\frac{-t^2}{2}}$$

agree with the theoretical might be, to a certain extent, fictitious. It will be noted that to the left of the mean the observations differ from the theoretical values by an excess, and to the right by a deficiency. In no case are the differences marked, and this leads to rather high values for the probability. However, if the consecutive positive and negative residuals are combined, the resulting number of classes is decreased from 12 or 14 to about 4 or 5. When tested by this highly unfavorable method of grouping, the probabilities are reduced to about 0.02. When both methods of testing are taken into account, it can be concluded that the observations check with the theoretical curves fairly satisfactorily but not as well as is indicated by the χ^2 test without grouping.

It should be observed that the theoretical curves extend from $-\infty$ to $+\infty$, but the observed frequencies necessarily must begin at zero. The area to the left of the zero point of the theoretical curves (normal) varies from 0.5 to 4 per cent of the total; with the corrected curves these values are even less. For exact analysis, theoretical curves that begin at zero should be used for comparison, but because of the relatively small part of these curves in the negative region, the more convenient strictly normal type of curve was used in this work. Finally, table 5 shows that two terms of a Charlier's series are as efficient in describing the populations as are three terms. The values of χ^2 are lower with three terms but the loss of an additional degree of freedom, because

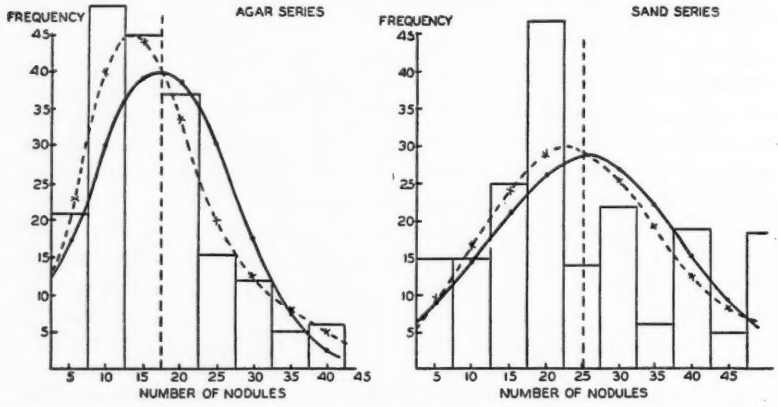

FIG. 2. COMPARISON OF THE OBSERVED DISTRIBUTION OF NUMBER OF NODULES ON CLOVER WITH TWO THEORETICAL CHARLIER'S SERIES

$\phi(t)$ is the normal curve of errors

●——● $F(t) = \phi(t)$

x---x $F(t) = \phi(t) - \frac{\alpha_3}{3!}\phi^3(t)$

$$\phi(t) = \frac{1}{\sqrt{2\pi}} e^{\frac{-t^2}{2}}$$

of the use of another moment in the specification of the theoretical curve, cancels the advantage of a lowered χ^2, and the probabilities remain much the same. This confirms the conclusion reached from the values of α_4, viz., none of the curves are decidedly peaked or flat. Since the second term of the Charlier's series corrects for asymmetry and the third term for flatness, it can be concluded that the only indication of a departure from normality in any of the curves is a slight asymmetry toward the lower values of the variables. The estimated values of the more important statistics for these distributions as well as those for the distributions of the other variables are given in table 6.

In actual practice, the data are seldom collected on *individuals* of the populations under consideration, but on samples from at least two, to as high as ten

TABLE 6

Maximum and minimum values; means, and standard deviations

	AGAR				SAND			
	Maximum	Minimum	Means	Standard deviation	Maximum	Minimum	Means	Standard deviation
Nitrogen fixed.....*mgm.*	3.24	0.16	1.13 ±0.03	0.44 ±0.02	14.27	0.33	5.56 ±0.22	3.18 ±0.16
Length of stem.....*cm.*	15.0	2.0	9.95 ±0.16	2.19 ±0.11	18.0	3.0	10.88 ±0.20	2.85 ±0.14
Length of root.....*cm.*	43.0	6.0	17.56 ±0.42	5.94 ±0.30	28.5	8.5	17.19 ±0.22	3.15 ±0.16
Number of nodules.....	51	4	17.50 ±0.68	9.57 ±0.48	100	4	28.56 ±1.24	17.68 ±0.88
Number of leaves.....	14	4	9.3 ±0.13	1.81 ±0.09	19	3	7.24 ±0.18	2.49 ±0.12
Ratio $\frac{\text{root}}{\text{stem}}$			1.82 ±0.04	0.60 ±0.03			1.71 ±0.03	0.49 ±0 02

plants. For this reason, the question in which we are particularly interested is the nature of the distribution of the *means* of a sample of n individuals from populations distributed as are those indicated in figures 1 and 2. Poincaré (7, p. 189–223) gives a demonstration which shows that even if the error functions of the individual errors are different from one another and from the normal Gaussian curve of errors, the sum of n errors will form a normal distribution, although n may be only moderate in size. The only assumptions necessary regarding the nature of the unknown error functions are that they are small, of like magnitude, and are even valued (negative errors are as likely as positive ones).[3]

[3] The same sort of a demonstration can be applied to our problem. Suppose a population forms a distribution that can be described by any function $f(t)$; what is the nature of the distribution of the *means* of samples of n individuals from this population? Without specifying the nature of $f(t)$, some idea of the distribution can be obtained by the calculation of the α's for the means of n individuals, $a_1, a_2, \ldots\ldots\ldots a_n$. The second moment of the distribution of the means (M_2) will be given by the expression:

$$M_2 = \int_{-\infty}^{\infty} \ldots\ldots\ldots \int_{-\infty}^{\infty} \frac{(a_1 + a_2 \ldots\ldots + a_n)^2}{n^2} f(a_1) \ldots\ldots\ldots f(a_n) da_1 \ldots\ldots da_n$$

Since the first moment of the *individuals* (μ_1) is taken to be zero,

i.e., $$\mu_1 = \int_{-\infty}^{\infty} a_i f(a_i)\, da_i = 0,$$

all cross product terms in the expansion of $(a_1 \ldots\ldots\ldots a_n)^2$ will disappear,

$$\therefore M_2 = \int_{-\infty}^{\infty} \frac{na_i^2}{n^2} f(a_i)\, da_i = \frac{1}{n}\mu_2$$

Where μ_2 is the second moment of the distribution of the individuals. Likewise the third and fourth moments can be calculated:

$$M_3 = \int_{-\infty}^{\infty} \ldots\ldots\ldots \int_{-\infty}^{\infty} \frac{(a_1 + a_2 \ldots\ldots a_n)^3}{n^3} f(a_1) \ldots\ldots f(a_n)\, da_1 \ldots\ldots da_n$$

$$= \int_{-\infty}^{\infty} \frac{na_i^3}{n^3} f(a_i)\, da_i = \frac{1}{n^2}\mu_3$$

$$M_4 = \int_{-\infty}^{\infty} \ldots\ldots\ldots \int_{-\infty}^{\infty} \frac{(a_1 + a_2 \ldots\ldots a_n)^4}{n^4} f(a_1) \ldots\ldots f(a_n)\, da_1 \ldots\ldots da_n$$

$$= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \frac{na_i^4 + 3n(n-1)a_i^2a_j^2}{n^4} f(a_i) f(a_j)\, da_i\, da_j$$

$$= \frac{\mu_4}{n^3} + \frac{3(n-1)}{n^3}\mu_2^2$$

From these moments, the values of the α's for the distributions of the means of n individuals can be calculated by the usual formulas in terms of the α's for the distributions of the individuals:

$$\bar{\alpha}_3 = \frac{M_3}{M_2^{3/2}} = \frac{\mu_3}{n^2} \cdot \frac{n^{3/2}}{\mu_2^{3/2}} = \frac{\alpha_3}{\sqrt{n}}$$

$$\bar{\alpha}_4 = \frac{M_4}{M_2^2} = \frac{\mu_4 + 3(n-1)\mu_2^2}{n^3} \cdot \frac{n^2}{\mu_2^2} = \frac{1}{n}\frac{\mu_4}{\mu_2^2} + \frac{3(n-1)}{n} = \frac{\alpha_4}{n} + \frac{3(n-1)}{n}$$

in which the bar over the α signifies that it belongs to the distribution of the means of n

Correlation coefficients

In many experiments, it is not always possible to make nitrogen determinations, nodule counts, etc. but some idea of the magnitude of these variables may be desired; this information may be approximated if the value of some other variable whose correlation with the variable not measured is known. Thus the correlation of one variable with another has practical application in the laboratory as well as theoretical interest in respect to the interrelationship between the various measurements of the growth of the plant. Both the simple and the multiple correlation coefficient among the several variables measured were calculated from the data; the results are given in table 7. The values of these estimations were tested for significance by calculation of the statistic t as described by Fisher (2, p. 159), and all were found to indicate real correlation except that between nitrogen fixed and $\frac{\text{length of root}}{\text{length of stem}}$ in the agar series. The observed correlation between the *number of nodules* and the *length of root* in the agar series is just on the limit of significance, as the probability that the observed correlation arose from random sampling is 0.05. The observed differences between the correlation coefficients for plants in agar and in sand were tested for significance by the calculation of z as described by Fisher (2, p. 163). This statistic is determined for each value of r by the formula:

$$Z = 1/2\,\{ln\,(1+r) - ln\,(1-r)\} = \log\frac{1+r}{1-r} \times 1.15$$

The difference between the z's corresponding to two values of r (r_1 and r_2) has a standard deviation equal to $\sqrt{\frac{1}{n_1-3}+\frac{1}{n_2-3}}$ (2, p. 170) where n_1 and n_2 are the number of pairs of values used in the estimation of r_1 and r_2. If the difference in the z's is less than twice its standard deviation, the observed difference in the r's is hardly significant. From the values given in column 8 of table 7, it is apparent that the observed values of corresponding simple correlation coefficients in the sand and agar series differ only in the following instances: (*a*) *nitrogen fixed* with *number of nodules*, (*b*) *nitrogen fixed* with $\frac{\text{length of roots}}{\text{length of stems}}$, and (*c*) *number of nodules* with *length of stem*. In all cases the correlation coefficient is lower in the agar series. Of the multiple correlation coefficients, it appears that the value of r for *nitrogen fixed* with *number of*

individuals. A consideration of the values of the $\bar{\alpha}$'s indicates that the f(t) may be a function that departs decidedly from the normal curve (as indicated by the value of the α's) but that *means* of even five individuals would tend to form a normal distribution. For example, if f(t) were such that the value of α_4 were only 2.0; the value of $\bar{\alpha}_4$ for means of five individuals would tend toward: $\frac{2.0}{5}+3\left(\frac{4}{5}\right)=2.8$, very close to the theoretical value of 3 for the normal curve.

TABLE 7

Summary of correlation coefficients

CORRELATION BETWEEN	AGAR SERIES			SAND SERIES			DIFF. IN z / S.D.$_z$*
	r	$\sqrt{1-r^2}$	z	r	$\sqrt{1-r^2}$	z	
Nitrogen fixed and number of nodules	0.39	0.920	0.4103	0.63	0.776	0.7384	3.25
Nitrogen fixed and length of stem	0.52	0.854	0.5747	0.63	0.776	0.7384	1.62
Nitrogen fixed and length of root	0.39	0.920	0.4103	0.25	0.968	0.2554	1.53
Nitrogen fixed and number of leaves	0.41	0.912	0.4454	0.56	0.829	0.6306	1.83
Nitrogen fixed and $\frac{\text{length of root}}{\text{length of stem}}$	−0.02	0.999	0.0203	−0.44	0.898	0.4704	4.46
Nitrogen fixed, number nodules, and length of stem	0.58	0.815	0.6595	0.71	0.704	0.8842	2.22
Nitrogen fixed, number nodules, and length of root	0.52	0.854	0.5747	0.64	0.768	0.7564	1.80
Nitrogen fixed, length stem, and length root	0.57	0.821	0.6450	0.64	0.768	0.7564	1.10
Number of nodules and length of stem	0.29	0.958	0.2983	0.56	0.829	0.6306	3.29
Number of nodules and length of root	0.14	0.990	0.1402	0.19	0.981	0.1919	0.51
Length of stem and length of root	0.34	0.940	0.3526	0.19	0.981	0.1919	1.59

* Standard deviation of the difference between z's = 0.101.

nodules and length of stem is significantly less in the agar series than in the sand series.

A consideration of the various coefficients of correlation shows that the values of r are positive except the one in the sand series between nitrogen fixed and $\frac{\text{length of root}}{\text{length of stem}}$; in this case high values of *nitrogen fixed* appear to be associated with low values of the ratio, i.e., more nitrogen is fixed by the stocky plants. Although the values of most of the estimations of the correlation coefficient are significantly different from zero, it cannot be claimed that the knowledge of the magnitude of one variable gives much information concerning

TABLE 8

Contingency tables for the type and distribution of nodules with nitrogen fixed and number of nodules (agar series)

TYPE AND LOCATION	TAPROOT		INTERMEDIATE ROOTS		TOTALS
	TR*	TL	IR	IL	
I. Nitrogen fixed *mg.*					
0.00–0.70	4 (2.7)	6 (8.1)	8 (6.4)	12 (12.8)	30
0.70–1.40	8 (9.7)	33 (29.8)	20 (23.5)	49 (47.0)	110
1.40–2.10	5 (4.6)	13 (14.1)	13 (11.1)	21 (22.2)	52
Totals	17	52	41	82	192
Test for independence	$X^2 = 3.30$ $n = 6$ Probability = 0.77				
II. Number of nodules					
0–20.5	11 (11.7)	39 (35.8)	20 (28.2)	62 (56.3)	132
>20.5	6 (5.3)	13 (16.2)	21 (12.8)	20 (25.7)	60
Totals	17	52	41	82	192
Test for independence	$X^2 = 10.51$ $n = 3$ Probability = 0.02				

* TR = taproot, round type; TL = taproot, long type; IR = intermediate roots, round type; IL = intermediate roots, long type; numbers in parentheses are theoretical values for cell.

the corresponding value of any other. This is indicated by the *coefficient of alienation* (8, p. 128) given in columns 3 and 6, table 7. The value of this statistic, $\sqrt{1 - r^2}$, is a measure of the improvement that can be made in the error of estimate through knowledge of the value of a correlated variable. For example, in the case of those two variables which showed the highest correlation, viz., *nitrogen fixed* and *number of nodules* in the sand series (0.63), the variability in the array of values of the number of nodules corresponding to a given value of the milligram nitrogen fixed is almost four-fifths of the average variability of *all values* of the number of nodules. Hence the correlation coefficient would aid primarily in the estimation of the *mean* number of nodules of a

large number of samples corresponding to a given value of nitrogen fixed. A comparison of the multiple with the simple correlation coefficients shows that measuring two variables gives little information with respect to a variable not estimated, in addition to that gained by the measurement of the single variable with the higher value of r. For example, the error in the estimate of the nitrogen fixed by a plant in agar is 0.44 mgm.; knowledge of the length of the stem would reduce this to $0.854 \times 0.44 = 0.376$ mgm., but the additional knowledge of the number of nodules would not help much, since in this case the error would be $0.815 \times 0.44 = 0.359$ mgm.

TABLE 9

Contingency tables for the type and distribution of nodules with nitrogen fixed and number of nodules (sand series)

TYPE AND LOCATION	TAPROOT		LATERAL ROOTS		TOTALS
	TR*	TL	LR	LL	
I. Nitrogen fixed mgm.					
0–4	7 (6.5)	15 (13.85)	5 (5.0)	49 (50.7)	76
4–8	6 (6.4)	13 (13.7)	6 (4.9)	50 (50.0)	75
>8	4 (4.1)	8 (8.5)	2 (3.1)	33 (31.3)	47
Totals	17	36	13	132	198
Test for independence	$X^2 = 0.98$ $n = 6$ Probability = 0.98				
II. Number of nodules					
0–20.5	10.5 (8.7)	16 (18.45)	7.5 (6.65)	68.0 (67.7)	101.5
20.5–40.5	4.5 (5.15)	14.5 (10.9)	4.5 (3.95)	36.5 (40.0)	60
>40.5	2.0 (3.15)	5.5 (6.65)	1.0 (2.4)	27.5 (24.3)	36.5
Totals	17	36	13	132	198
Test for independence	$X^2 = 4.33$ $n = 6$ Probability = 0.63				

* TR = taproot, round type; TL = taproot, long type; LR = lateral roots, round type; LL = lateral roots, long type; numbers in parentheses are theoretical values for cell.

For the estimation of association between variables of which one or more are capable only of qualitative measurement, the use of the contingency table is of value. In the analysis of the data on the number of nodules on plants in sand and in agar two striking differences were encountered, viz., much higher numbers of nodules in the sand series, as well as the appearance of a second mode in the frequencies of the nodules. It was believed that these differences might be associated with the location of the nodules on the roots. The type and location of the nodules was estimated in a qualitative manner by classifying the nodules on each plant into: (*a*) round or long type (*b*) predominately located on the tap, intermediate, or lateral roots. It is realized that a classification of this type can be at best only approximate, since a great deal depends on the

personal bias of the observer, but in spite of these limitations, the use of this description of the location and type of nodules has proved of value in many experiments. Examination of the original data showed that the invasion of the lateral roots was almost negligible on the plants grown in agar, but that this type of invasion ranks very high on plants grown in sand. For example, in agar, 69 of the 196 plants examined showed nodules primarily on the taproot, 123 on the intermediate roots, and only 4 on the lateral roots. In sand, however, 53 of the 202 plants had nodules predominately on the taproot, 4 on the intermediate roots, and 145 on the lateral roots. It seems likely that the difference between the numbers of nodules is connected with the differences in the location. In order to determine whether the type and location of the nodules were associated with nitrogen fixed or with the number of nodules the contingency tables 8 and 9 were prepared and tested for independence by the X^2 test (2, p. 82). Whenever the location of the nodules on a plant was not definitely in one class, it was divided into 0.5 units for classification as shown in table 9. In both the sand and agar series, the values of the probabilities found are very high that there is complete independence between the *type and location of the nodules* and *nitrogen fixed*; this is likewise true for the sand series between the *type and location of nodules* and *the number of nodules*. However in the agar series, there is evidence that there is some association between the *type and location of nodules* and *the number of nodules*. Upon examining the individual values of the X^2 contributed by the various cells in the table, it appears that the indicated association is due to an excess of the round type of nodule on the intermediate roots of those plants having more than 20.5 nodules. In order to investigate this further and to determine whether there is any masking of association between two of the variables in a large table of this type, the data given in tables 8 and 9 were reassembled in four-fold and six-fold types of contingency tables and the following variables tested for association (12, p. 378):

Location of nodules with number of nodules.
Type of nodules with number of nodules.
Location of nodules with type of nodules.
Location of nodules with nitrogen fixed.
Type of nodule with nitrogen fixed.

In both the sand and agar series, complete independence of the foregoing pairs of variables was found except that in the agar series the long type of nodules was associated with the lower number of nodules, and in the sand series, there was an excess of the round type of nodules on the taproot.

SUMMARY

Inoculated clover plants were grown on agar in cotton-plugged bottles and on sand in open jars in the greenhouse and the following measurements taken: milligrams nitrogen fixed; number, type, and location of the nodules; length of stems and roots; and number of leaves.

From these data, a statistical analysis was made which included the following tests and calculations: homogeneity of the data on milligrams nitrogen fixed and on the number of nodules per plant; distribution curves of the nitrogen fixed and the number of nodules; simple and multiple correlation coefficients among the variables capable of quantitative estimation; and association tests among those variables which admit of only qualitative specification. The statistical methods used are discussed in detail.

The statistical tests of the data showed, that under the conditions of the described experiments, the populations sampled, for example, milligrams nitrogen fixed, are homogeneous with the exception of the number of nodules in the sand series. However, even in this case, the non-homogeneity is not marked. Likewise, the distribution curves of the variables examined are essentially normal except that of the number of nodules in the sand series. This observed departure from normality appears to be due to inaccuracies in counting the nodules rather than to any gross abnormalities in the type of distribution. A mathematical demonstration is given that shows normal statistics can be applied with confidence to the populations investigated (milligrams nitrogen fixed and number of nodules) if a few plants are combined for a measurement.

The simple correlation coefficients indicate that no two of the variables measured are very highly correlated with each other. In the sand series the greatest correlation was observed between the number of nodules and the milligrams of nitrogen fixed (0.63) and the length of stem and milligrams of nitrogen fixed (0.63). In agar, the highest correlation coefficient was between the length of the stem and milligrams nitrogen fixed (0.52); the correlation between the number of nodules and milligrams nitrogen fixed was only 0.39. Multiple correlation coefficients in which two variables were correlated with milligrams nitrogen fixed were only slightly better than the higher of the two simple correlation coefficients between milligrams nitrogen fixed and these variables.

The following pairs of variables were tested for association in both the agar and sand series: location and type of nodules with number of nodules and with milligrams nitrogen fixed; location of nodules with type of nodules. Association was indicated only between type of nodules and number of nodules in agar and between type of nodules and location of nodules in sand.

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RELATION BETWEEN CARBON DIOXIDE AND ELEMENTAL NITROGEN ASSIMILATION IN LEGUMINOUS PLANTS[1]

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Two of the most important chemical reactions in nature are the assimilation of carbon by higher plants and the assimilation of elemental nitrogen by microörganisms. It is a commentary upon the economy of nature that these two life-supporting syntheses should occur simultaneously in one family, viz., the *Leguminosae*. Both processes have been studied independently of one another, but little work has been done with the view of determining the interrelation between them. Such studies might be made in two ways: by a comparison of the carbohydrate and nitrogen metabolism of plants fixing nitrogen with others grown under similar conditions but supplied with combined nitrogen; by modifying one process, e.g., photosynthesis, and observing the effect on the other. During the past year studies have been started on the problem of the relationship between carbon assimilation and nitrogen fixation in *Leguminosae* by use of the second method of attack; the results are given in this report.

Modification of the carbon assimilation process might be accomplished in a variety of ways: change of temperature, pressure of CO_2, intensity or quality of light. Nitrogen fixation may be influenced: by the addition of a source of combined nitrogen, e.g., nitrates, by the use of different strains of rhizobia, and by other factors. Since many of the factors concerned with nitrogen fixation are not known, it appeared advisable to limit the first experiments to changing the photosynthetic activities of the plant rather than attempting to control the nitrogen fixation. Later, experiments will be made in which both carbon assimilation and nitrogen fixation will be modified. The variable factor controlling photosynthesis that can be most easily regulated in greenhouse experiments is the partial pressure of CO_2 in the atmosphere supplied to the plants; therefore, this variable was selected for study. Specifically, plants were supplied with atmospheres containing different partial presssures of CO_2 and the effects on nodule formation, nitrogen fixation, dry weight, and the general anatomy of the plant were determined.

It is not feasible in this paper to attempt a complete review of the literature concerned with the effect of increased CO_2 supply on the growth of plants. The monographs of Reinau (15), Fischer (6), Bornemann (3, 4), and Lundegardh (12) contain excellent historical reviews of this subject; and less detailed accounts are given in Spoehr (19), Stiles (20), and Niklas, Scharrer, and Strobel (13). However, it will be advantageous to consider briefly certain points which deal with the historical and practical aspects of the subject, before the more detailed review of the literature concerned with the leguminous species is made.

HISTORICAL

In 1804, de Saussure (18), grew young pea plants in atmospheres containing 8 to 100 per cent CO_2, and exposed to bright or diffuse sunlight. In the diffuse light, all concentrations of

[1] Herman Frasch Foundation in Agricultural Chemistry, Paper No. 47, Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

CO_2 were detrimental to the plant; but in the full sunlight, the plants receiving 8 per cent CO_2 showed better growth than the controls grown in ordinary air. Albrecht Thaer (21), in 1837, emphasized that the value of the use of organic manures was partially due to the CO_2 liberated by their decomposition.

Krueseler (10) in 1885, grew plants in mixtures of carbon dioxide and air ranging from 0.03 to 12 per cent. He used an arc light of 1,000 candle power in order to control the intensity of light during the experiment; this light source was placed 30 to 45 cm. from the plants and was separated from them by a water screen. Kruessler found that the assimilation of CO_2 was dependent on the partial pressure of CO_2 in the atmosphere. As the pCO_2 was raised from 0.03 to 1.0 per cent the increase in assimilation was quite rapid but became less rapid from 1 to 6 per cent; finally an increase in the pCO_2 above 6 per cent had no further effect on the assimilation.

Demoussy (5) grew a number of ornamental plants in closed glass boxes and supplied them with an atmosphere which contained about 0.2 per cent CO_2; the plants receiving CO_2 had dry weights of 2 to 5 times those of the controls which were supplied with ordinary air. Since 1910, a great deal of work has been done in Europe in regard to the practical aspect of addition of CO_2 to plants in greenhouse and field; the feasibility and technique of the so-called "Kohlensäuredüngung" has engaged the efforts of a large number of investigators, notably Reinau, Lundegardh, Bornemann, Fischer, Klein, and Riedel. The contributions of these workers will be discussed in subsequent sections.

Sources of CO_2

The pioneer work of de Saussure (18), Kruessler (10), and others demonstrated that the dry weights of plants could be materially increased by the addition of CO_2 to the atmosphere. The investigations of the later workers have dealt with practical methods for increasing the supply of CO_2 and more detailed analysis of the effects. Before considering the results of the latter studies, it might be of interest to review some of the proposed methods for increasing pCO_2 in atmospheres supplied to plants. The methods are conveniently divided into the inorganic and the organic, or more properly, biological. The obvious example of the first type is the use of compressed CO_2 in tanks or by the liberation of CO_2 from $CaCO_3$ with acids. These methods are certainly the most convenient for experimental work and are used almost exclusively in theoretical studies. In addition, some experiments have been made in both greenhouse and field which indicate that the judicious use of CO_2 in cylinders in these places might be economical.

A second type of the inorganic method is CO_2 production by burning coke, charcoal, or alcohol. Reinau has patented a burner called the *OCO Dunggasspender* which burns a standard coke briquette; this produces a known quantity of CO_2, so that in a closed space, e.g., a greenhouse, the pCO_2 can be regulated to a certain extent. The burner is so constructed that it produces little heat and no poisonous gases; hence it can be used without purification apparatus. Riedel (16) has patented a larger type of CO_2 producer from which the gas is piped to greenhouse or field; he has also purified gas from blast furnaces and used it as a source of CO_2 in greenhouse and field experiments. Niklas, Scharrer, and Strobel (13) describe a patented mixture of the composition: peat, 50 per cent; wood charcoal, 45 per cent; MnO_2, 5 per cent; this apparently benefited certain plants when applied at the rate of 350 to 1,070 pounds per acre.

Turning to the organic or biological methods, we find that one of the favorite proposals is to add peat, lignite, or other sources of carbon to the soil often accompanied by inoculation with bacteria which are known to oxidize carbon. In Germany during the war, a product called *Guanol*, which apparently is a mixture of peat with spent fermentation liquor, was used extensively. The effects of this fertilizer on crop yields were greater than the mineral content warranted and the additional action was ascribed to a slow fermentation which furnished a source of CO_2 to the plants. Other commercial products of this nature include *Humit*,

Bayerischer.—Kohlensäuredünger, and *Humuskohle*, In England, Bottomley suggested the use of a similar fertilizer called *Humogen* which was peat inoculated with bacteria; the results with this product are conflicting.

Reinau describes a greenhouse which was supplied with CO_2 from the fermentation of manure; water was sprayed over the manure and after the ammonia was scrubbed out, the fermentation gases were led over the plants. In this connection the CO_2 from the respiration of soil bacteria is probably very important in the natural CO_2 fertilization. This must be expecially true when the soil is high in organic matter, i.e., after the addition of stable or green manures. Lundegardh considers this source of CO_2 as one of the most important from a practical point of view, and he is inclined to the belief that it is the sole feasible, economical way of increasing the CO_2 in the atmosphere over a field. He has shown that over an average oat field, two-thirds of the soil respiration is due to soil bacteria and one-third to the root system of the plants. The CO_2 production is at a maximum in those fields which are high in organic matter, well aerated, and supplied with ample mineral salts and moisture.

The respiration of plant roots also can not be disregarded as a means of increasing the pCO_2 in the atmosphere about the plants. The rate of respiration is on the average only about one-seventh that of photosynthesis, but since plants respire CO_2 24 hours and assimilate only 6 hours a day, it follows that about one-half of the CO_2 assimilated during a day is respired and once more takes its place in the carbon dioxide cycle (12).

Results in practice

An adequate review of all the results obtained in greenhouse and field experiments is neither possible nor desirable for this paper, but a few of the important experiments will be discussed briefly. Reinau (16) furnished his *OCO Dunggasspender* to a large number of gardners and horticulturists, and requested testimonials in regard to its value for greenhouse and hotbed use. Even after discounting the lack of control in these experiments, the results appear to be overwhelmingly in favor of CO_2 fertilization. Bornemann (4) describes experiments which indicate that the entrance of the different growing forms, viz., vegetative, blooming, fruiting, and storage of reserves are all hastened by supplying plants with CO_2. Harder, Keppler, and Reusz (8) investigated the effect of added CO_2 and artificial light on plants grown in the greenhouse during the winter. With several species of flowers, they demonstrated that excellent and early blooms could be induced by the light plus CO_2; less striking results were obtained with light or CO_2 alone. With strawberries, ripe fruit was produced during the winter months only when both CO_2 and artificial light were used. Fischer (6) has also demonstrated that earlier blooming and fruiting can be induced in a number of plants by increasing the CO_2 in the atmosphere in which they are grown; there is also some evidence that resistance to diseases is increased in plants supplied with increased CO_2.

In field experiments the results have been encouraging in spite of numerous technical difficulties. Riedel (16, 17) purified gas from blast furnaces, piped it into fields growing barley, lupines, and potatoes, and obtained increase of 50 to 190 per cent in the dry weights of the plants so treated over those of the controls. Larger leaf surface, earlier blooming, and a greater yield of fruit were typical differences noted in the plants receiving CO_2. Bornemann (3) used CO_2 from cylinders in field experiments and found increases of 25 to 210 per cent in the yield when compared with the control plants. He also used *Guanol* as a source of CO_2 in field experiments and obtained very remarkable increases in the yield of peas, turnips, mustard, and strawberries. However, with Bottomley's *Humogen*, the results were negative. Niklas et al. (13) used a patented peat-charcoal MnO_2 mixture as a source of CO_2 and obtained increases in 7 out of 12 trials. Lundegardh (12) criticizes the results of Fischer and Bornemann on the ground of the lack of proper controls made necessary through differences in the fertility of the plots used. He also objects to the use of high concentration of CO_2 in field trials, arguing that this is not feasible in actual practice. In his own experiments, the CO_2 was supplied at about the same rate at which it would be liberated from the soil; for example, in one trial,

10,000 liters of CO_2 per 100 sq. m. were supplied to turnips during the growing season at the rate of 0.4 cc. per minute. As much as 42 per cent of the CO_2 added was recovered in the crop.

Effect of CO_2 on leguminous plants

In reference to our own experiments, the effects of added CO_2 on leguminous plants are of special interest, therefore the available data will be considered in greater detail than was possible in the foregoing general section. In the reports made to Reinau (15) on the efficacy of his *Dunggasspender*, practical gardeners state that leguminous plants gave increased yields of 50 to 100 per cent when supplied with CO_2. Riedel found that beans grown with additional CO_2 had a dry weight of 1.5 that of the controls with 5.6 times as many blooms and 5 times as many nodules. Lundegardh observes that leguminous plants are especially "thankful" for CO_2 fertilization; in a greenhouse experiment, he found that by increasing the concentration of CO_2 51 per cent, an increase in the yield of beans of 112 per cent was obtained. In similar experiments in which the concentration of CO_2 was varied from 0.03 to 0.08 per cent, it was observed that the response of beans to an increase in the pCO_2 was much greater than was that of cucumbers and tomatoes. Lundegardh also reports that beans grown in an atmosphere containing 0.08 per cent CO_2 had 10 per cent more chlorophyll in the leaves than did beans grown in air.

Cummings and Jones (4a) report that both beans and peas when treated with CO_2 gave higher yields than did the untreated controls; the gassed samples are lower in protein, i.e., more carbohydrate material is formed.

Niklas et al. (13) grew red clover in soil to which a patented peat mixture was added and found slight losses in the dry weights when compared with the controls, whereas gains were noted with barley, oats, potatoes, and field beans. Vouk (23), likewise, could not obtain any evidence of beneficial action when lignite or peat was added to soil used to grow peas, vetch, and beans, although radishes, *Tradescantia*, lettuce, and tomatoes were markedly benefited. The higher concentrations of the lignite or peat were toxic to the leguminous plants although with vetch an increase in the number of nodules was noted; however, with beans a decrease of nodules was observed. Vouk was inclined to the belief that the effect of the lignite or peat was dependent on its nitrogen content. Lieske (11) disputes this and cites experiments of his own in which a "rough lignite" was treated with ammonium salts and added in very small quantities to the soil (1 to 50 gm. per 30 kgm. of sand plus soil). In all cases a beneficial action was noted which was independent of the nitrogen added. He thinks Vouk's evidences of toxicity were due to large quantities of lignite added (10 to 100 parts per 100 parts soil); in Lieske's experiment both bush-bean and lupines gave increased yields when treated with the lignite preparation. However, Lieske attributes the activity of the lignite to its colloidal nature, which aids the plant in its nutrition by modification of the permeability of the plasma membrane; he believes other effects, e.g., CO_2 production, are secondary.

Arthur, Guthrie, and Newell (2) found that the addition of CO_2 and the use of artificial light were very effective in hastening the growth of soybeans and clover. The clover was grown from seed to flower in 38 days provided 18 hours of light were used in conjunction with 0.3 per cent CO_2. With CO_2 and added light the dry weight of the plants was 2 to 8 times that of the controls; most of this increase was in the carbohydrate fractions as the ratio $\frac{\text{carbohydrate}}{\text{nitrogen}}$ was about 4 for the control plants and 12 for the treated. Similar results were obtained with soybeans. In neither case was there any mention of nodules, and it appears that the plants were furnished combined nitrogen.

In general, the results indicate that the *Leguminosae* are decidedly responsive to an addition of CO_2; the negative results reported are likely due to the method used to supply this gas. Reinau (14) adds other evidence that sheds light on the importance of increased CO_2 supply to leguminous plants. He observed that the respiration of soil bearing clover or seradella is

much higher than is that of soil cropped to rye or mustard; also the respiration of the soil between rows of clover is only about 50 per cent of that near the plants. If the tops of the legumes are removed, however, the soil respiration decreases 33 to 50 per cent, whereas with non-legumes the decrease is only 5 to 10 per cent. He also observed that the CO_2 content of the air near the soil of fields planted with leguminous species is much higher than is that of the air near the tops of the plants. Reinau interprets these data as indicative of a high rate of respiration by the bacteria in the nodules of the plants; this respiration liberates sufficient CO_2 to increase the pCO_2 in the atmosphere surrounding the lower leaves and these leaves are thus able to assimilate CO_2 in spite of the lower light intensity available to them. It appears that a miniature CO_2 cycle characterizes the development of legumes: CO_2 is assimilated by the upper leaves; the carbohydrate formed is partially respired by the bacteria in the nodules; and the CO_2 liberated is resynthesized by the lower leaves.

In this connection, Kostytschew (9) has shown that the rate of CO_2 assimilation per unit leaf area is approximately the same for most species except the *Leguminosae*, which have much higher rates. He believes that this difference is related to the nitrogen supply of the plants but his evidence is not too convincing. It is of interest that *Alnus*, which also forms nodules, has a rate of assimilation about equivalent to that of the non-leguminous species. Connected with this high rate of assimilation and intensifying it in actual practice is the large leaf surface per unit of soil occupied by legumes as compared with other species. Bornemann (3) cites data which show that the leaf surface per square meter of soil for alfalfa and clover is much higher than for rye, oats, barley, and wheat.

In conclusion, it is to be noted that the experiments dealing with the effect of CO_2 on the growth of leguminous plants have in general been made from the point of view of CO_2 fertilization, and little attention has been given to the nitrogen fixation aspect. In most of the papers reviewed, it was not even stated whether the plants were inoculated or supplied with combined nitrogen. For these reasons, it is believed that a more detailed study of the effects of the addition of CO_2 to legumes is desirable and that such studies should yield valuable information in regard to the nitrogen fixation process.

THE EFFECT OF CO_2 ON NITROGEN FIXATION BY CLOVER AND ALFALFA

The experiments described in this section were made with clover and alfalfa plants grown in bottles in the greenhouse under various partial pressures of CO_2. The technique for growing the plants has been given in detail in another publication (24). A few methods used in the chemical analyses of the plants will be described in this paper.

Methods

Dry weight. The plants were washed from the sand or removed from the agar with large forceps and placed on filter paper in a 37°C. incubator which was provided with a large fan to keep the air circulated. After 48 hours, the plants were transferred to a $\frac{1}{4}$-pint milk bottle, capped, and kept in the incubator until ready for analysis. The dry weight, to the nearest 5 mgm., was determined on an analytical balance.

Total nitrogen. Total nitrogen in the absence of nitrates was determined by the official Kjeldahl method; in the presence of nitrates the salicylic acid modification was used.

Sand. Great difficulty was experienced in removing the sand from the roots, in spite of long washing with tap water. Since the percentage of nitrogen in

the plant is used as a criterion of the effect of the added CO_2, it is necessary that the dry weight be fairly accurate; this is impossible unless the sand is estimated in some manner. The following method was developed for this purpose and proved very satisfactory. The plants are removed from the sand, the roots washed well in flowing tap water, then soaked in 20 per cent NaCl solution for 10 minutes, followed by a second washing in tap water. This procedure removes most, but not all of the adhering sand. The plants are dried, weighed, and digested for the nitrogen determination; the digestion allows the sand present to collect at the bottom of the Kjeldahl flash, where after dilution with 250 cc. distilled water, it can be estimated by a comparison with "sand standards." These standards are made by adding a known weight of sand to about 1 gm. of sand-free plant material, digesting with H_2SO_4, etc., and finally diluting with 250 cc. distilled water. The standards used contained 25, 50, 100, 150, 250, and 300 mgm. of sand; with these standards, the sand in an unknown can be estimated with a maximum error of 10 to 15 per cent. Since the dry weight of plant material is about 10 to 30 times that of the sand present, the maximum error in the corrected dry weight of the plants is about 1 per cent. For example, with careful washing, the sand on the roots can be reduced to less than 150 mgm. per 10 plants; this quantity can be estimated by the method within 15 to 20 mgm. The dry weight of 10 plants is 1 to 1.5 gm., hence the error in the estimated weight becomes negligible. The method was tested by determining the sand in a number of unknowns which contained 10 to 275 mgm. of sand. The estimations were made by two individuals independently of each other, with results that checked each other and the true values within the indicated limits of accuracy.

EXPERIMENTAL

Experiment 1

As a preliminary experiment, red clover was grown on sand in 32-ounce bottles, supplied with an atmosphere containing about 0.5 per cent CO_2 and the nitrogen fixed compared with control plants grown in bottles closed with a cotton plug. Six plants were placed in each bottle and both sets were inoculated with *Rh. trifolii* 209, a strain that is known to fix considerable quantities of nitrogen in association with the host plant. The technique for preparation of the atmospheres was not developed at this time, and as a result the percentage of CO_2 in the mixture varied considerably. The plants were placed in the greenhouse on August 14, 1930; 14 days later those to receive CO_2 were connected to the manifolds. The plants were analyzed 72 days after planting. At this time those receiving CO_2 had completely filled the bottles, whereas the inoculated controls in the cotton plugged bottles were very much smaller. The nitrogen fixed per 10 plants by those supplied with CO_2 was 5.7 mgm. (11 samples) as compared with 1.2 mgm. (7 samples) fixed by the plants grown under a cotton plug.

The experiment was repeated except that the plants were grown on agar.

The cultures were planted November 8, 1930, but the growth was slow; on December 1, artificial lighting was started and there was noticed an immediate improvement in the plants, especially those receiving extra CO_2. The plants were analyzed after 58 days; at this time, those treated with CO_2 were tall, sturdy, and showed some signs of carbohydrate excess (red stems), whereas those grown in the cotton plugged bottles were pale and slender. The analyses are given in table 1, experiment 1. The statistical constants were calculated by the usual formulas and serve to give some idea of the variation among the individual samples. The data show conclusively that supplying clover plants grown in agar with CO_2 results in a pronounced increase in the quantity of nitrogen fixed; however, there is a greater increase in the dry weight of the plant so that the percentage of nitrogen in the plant actually decreases.

Experiment 2

The preliminary experiments indicated that the nitrogen fixation process in clover was partially dependent on the photosynthetic activities of the plants, but further experiments were believed necessary to verify this conclusion. Two questions required immediate answers before the problem could be further investigated, viz., (*a*) would the beneficial effects of the CO_2 be observed during seasons in which light intensity was not limiting photosynthesis by the plants; (*b*) how much of the benefit noted could be ascribed to aeration *per se*, independent of the added CO_2? In order to investigate these questions, experiments were made during the spring and summer of 1931 in which additional controls were included; these controls were supplied with air containing 0.03 per cent CO_2 at the same rate as were the cultures aerated with the 0.5 per cent CO_2 mixture. Plants on agar were set up May 21, 1931, inoculated with *Rh. trifolii* 209 and placed in the greenhouse. These were connected to the manifolds 14 days later; the growth was so rapid in spite of a heat wave during June, that 46 days after planting the plants were ready for analyses.

The plants which received CO_2 were dark green with red stems (carbohydrate excess), thrifty, sturdy, and inclined to be pubescent. These plants had a large number of long finger-like nodules on, or near, the taproot and also a number of small, round scattered nodules. The appearance of the nodules was very striking; many were abnormally large and very pink. There did not appear to be any correlation between the number of nodules and the appearance of the plants, except that those plants which had the most numerous and largest nodules did not show so much evidence of carbohydrate excess. The plants receiving air were light green, smooth, and spindly, with no signs of carbohydrate excess. The nodules were long and located for the most part on the taproot near the crown. These plants were no better than the plants grown in bottles closed with cotton plugs; this was very likely because of the rapid growth of the plants receiving CO_2, which made it necessary to analyze all the plants before any difference in the two sets of controls could manifest itself.

The results of the analyses are given in table 1, experiment 2, agar series. The average length of the tops and roots gives some idea of the relative stockiness of the plant; the results show that the plants receiving CO_2 were much

larger than the others, with a short, heavy root system. The increase in the number of nodules was 3 to 4 fold when CO_2 was supplied. As noted in experiment 1, there was an enormous increase in the nitrogen fixed but, since even this increase did not keep pace with the dry weight accumulation, the percentage of nitrogen in the plants receiving CO_2 showed a significant decrease.

At about the same time as the agar series was set up, the experiment was duplicated by a series in which sand plus modified Crone's solution was used as a substrate for the plant. The growth of the plants receiving CO_2 was again so rapid that it was necessary to make analyses at the end of 34 days;

TABLE 1

The effect of additional CO_2 on the growth and nitrogen fixation by clover plants

Experiments 1 and 2

TREATMENT	NUMBER OF SAMPLES	LENGTH OF TOPS	LENGTH OF ROOTS	NUMBER OF NODULES	DRY WEIGHT*	NITROGEN†	
						Total	Per cent
		cm.	*cm.*		*mgm.*	*mgm.*	
Experiment 1:							
Agar series—58 days:							
Air plus CO_2	9	....	....	.....	502 ±17	12.60 ±0.40	2.49 ±0.04
Cotton plug	9	....	....	.....	103 ± 4	3.80 ±0.20	3.69 ±0.14
Experiment 2:							
Agar series—46 days:							
Air plus CO_2	8	14.0	7.0	290 ±18	879 ±72	21.30 ±1.70	2.43 ±0.13
Air	4	8.2	7.0	80 ±16	153 ±20	5.25 ±0.92	3.43 ±0.20
Cotton plug	4	9.6	6.8	100 ± 5	186 ± 4	6.46 ±0.25	3.47 ±0.11
Sand series—34 days:							
Air plus CO_2	5	13.0	6.5	83 ±13	536 ±87	14.80 ±2.60	2.76 ±0.06
Air	5	8.4	7.0	34 ± 5	120 ±13	3.41 ±0.26	2.84 ±0.11
Cotton plug	3	8.8	7.0	35 ± 4	121 ± 2	3.64 ±0.20	3.01 ±0.09

* All figures in the tables are for 10 plants.

† These data are total nitrogen in the plants; uninoculated controls in agar contained 1.8 mgm. nitrogen; in sand, 2.0 mgm. of nitrogen.

and, as in the agar series, the plants receiving air alone did no better than those grown under cotton plugs.

The plants supplied with additional CO_2 were deep green, sturdy, and showed no signs of carbohydrate excess. The nodules on these plants were very large but not nearly so numerous as in the agar series; their color was pink to gray, and they were located on the taproots. Some of the largest plants had only 5 to 6 nodules per plant, but these nodules were abnormally large. The plants receiving air and those grown under a cotton plug were smaller, paler in color, and much less stocky than the CO_2 treated samples. They possessed very few nodules, but those present were fairly large.

The analyses are given in table 1, experiment 2, sand series. In general, the results confirm those obtained with the plants grown on agar with one

outstanding exception, viz., there is no significant difference in the percentage of nitrogen in the plants.

Experiment 3

The results of the first two experiments definitely established that the quantity of nitrogen fixed by clover can be greatly increased if the photosynthetic activities of the plant are accelerated. In experiments 1 and 2, the CO_2 was added to the air by regulating the flow of bubbles through a tell-tale fitted with a 1-mm. capillary tube; since this method of control was not very exact, the CO_2 content of the air fluctuated greatly. Further experiments were deemed necessary to analyze in greater detail the effects of CO_2 on the nitrogen fixation process under more controlled conditions. Since in the first experiment, the CO_2 content of the air was the same for all plants receiving the air plus CO_2 mixture, it was decided to investigate the effect of different pCO_2 in the atmospheres supplied the plants.

On July 21, 1931, red clover was planted on sand in 64-ounce bottles; the larger bottles were used in this and subsequent experiments in order to allow more room for the growth of the plants. Eight hundred grams of sand plus 135 cc. of modified Crone's solution were placed in each bottle, in which 10 seedlings were planted and then inoculated with *Rh. trifolii* 209. Eight to ten of these cultures were connected to manifolds which supplied atmospheres of the following CO_2 content; air (0.03), 0.1, 0.2, 0.4, and 0.8 per cent. In addition to the usual cotton plugged controls, which represent plants growing in an atmosphere with a CO_2 content less than 0.03 per cent, a few bottles were left open to the air. It has been long observed that plants grown in a closed system never develop as well as plants grown in air; the factors concerned in this are probably differences in the quality and intensity of the light received and differences in transpiration and respiration. However, the appearance of some of the plants receiving CO_2 in the preliminary experiments compared very favorably with plants grown in open pots. In this experiment, a direct comparison between plants grown in the open with those in a closed system was made possible by the inclusion of the "open" controls.

Periodic analyses showed that the percentages of CO_2 in the gas mixtures remained within 5 to 10 per cent of the values desired, e.g., the CO_2 in the atmosphere that was supposed to contain 0.2 per cent of this gas fluctuated between 0.18 and 0.22 per cent. Analyses of the air supplied the plants and that in the greenhouse indicated that the CO_2 content varied between 0.025 and 0.035 per cent. The plant cultures were attached to the manifolds immediately after planting and were supplied with the various mixtures at the rate of 80 cc. per minute per bottle. The weather was excellent during the period of growth with few cloudy days and with no extended periods of intense heat. The plants were removed for analysis 41 days after planting.

0.8 per cent CO_2. These plants were dark green, thrifty, and pubescent. There was little sign of carbohydrate excess and the leaves were very large. There were many nodules on the

plants both large and small; the long type was clustered near the crown on the taproot with the small, round variety scattered among the secondary roots. The nodule distribution was characteristic of plants inoculated with poor strains of *Rh. trifolii*, Allen and Baldwin (1), indicating that the addition of CO_2 had changed the usual distribution of the nodules.

0.4 per cent CO_2. The plants receiving 0.4 per cent CO_2 were little different in appearance from those supplied with 0.8 per cent of this gas. Several showed more carbohydrate excess, but the luxuriance of development was about the same. The distribution and size of the nodules were practically the same as in the 0.8 per cent series except there was less tendency for invasion of the secondary roots.

0.2 per cent CO_2. The plants in this series were smaller and had fewer leaves than those receiving higher percentages of CO_2. The majority of the nodules were long and finger-like and were on the taproot near the crown.

0.1 per cent CO_2. These plants were similar to the 0.2 per cent series except they were not quite so large and had fewer leaves. The nodules were medium to large in size and were found on the taproot.

Air (0.03 per cent CO_2). The plants supplied with air were healthy and thrifty in appearance but not nearly so large as the plants receiving additional CO_2. Especially noticeable were the smaller leaves on these plants when compared with the other series. The size and distribution of the nodules differed little from the 0.1 per cent series and were typical of plants inoculated with a good strain of the organism.

Cotton plugs. In this series the plants were pale green, much smaller than any of the others, and spindly. The nodules were small and were found on the taproot.

Open to air. Growth of the plants in the bottles open to the air was excellent but no better than in the closed bottles receiving increased CO_2; the plants were sturdy, healthy, and dark green. The nodules were predominately on the taproot with some scattered on the secondary roots.

The data on the analysis of the plants are summarized in table 2, experiment 3. The increased growth of the plants receiving CO_2 is shown by the data on the length of tops and roots. There is a noticeable difference in the size of the plants supplied with air and those which received 0.1 per cent CO_2, but additional CO_2 did not appear to cause a corresponding increase in size. This is possibly because the closed container limits the height which a plant can reach. The number of nodules per 10 plants was doubled as the CO_2 content increased from 0.03 to 0.1 per cent and was increased 3-fold in the higher concentrations of CO_2. The response to increased CO_2 in the atmosphere is strikingly shown by the data for dry weight and nitrogen content of the plants. As the CO_2 supplied was increased, both the dry weight and total nitrogen of the plants increased, but there was a large variation in the response of the individual plants of a given series as indicated by the large standard deviation of the means.

Obviously, there are striking differences between the plants receiving CO_2 and those supplied with air alone, but it is difficult to decide whether or not the differences noted among the four series treated with different pCO_2 are significant. In order to determine whether the observed differences are real or only apparent, the results from the series receiving additional CO_2 were subjected to an analysis of variance (7, p. 196). The statistical test consists essentially in combining populations receiving different treatments and examining the resultant population for homogeneity. If the population is non-

homogeneous when tested by the appropriate method (z-test 7, p. 194), the observed differences between various treatments are probably significant. The results of this analysis showed that in every case, the treatments had given rise to a real difference in all of the variables measured. The differences in the means of the variables (dry weight, number of nodules, etc.) were further tested in pairs for significance by the modified "Student" method (7, p. 107).

The results are summarized in table 3 in the form of the probability that the difference noted between any two means could have arisen from random sampling; unless this probability is 0.05 or less, the difference cannot be held

TABLE 2

The effect of the pCO_2 on the growth and nitrogen fixation by clover plants

Experiments 3 and 4

TREATMENT	NUMBER OF SAMPLES	LENGTH OF TOPS	LENGTH OF ROOTS	NUMBER OF NODULES	DRY WEIGHT	NITROGEN	
						Total	Per cent
		cm.	*cm.*		*mgm.*	*mgm.*	
Experiment 3, sand—41 days:							
0.8 per cent CO_2	8	12.6	10.5	254 ±23	885 ±85	22.33 ±2.15	2.53 ±0.03
0.4 per cent CO_2	8	14.5	9.8	264 ±25	740 ±92	19.44 ±2.24	2.65 ±0.08
0.2 per cent CO_2	10	13.6	7.4	195 ±13	585 ±69	16.90 ±1.71	2.89 ±0.07
0.1 per cent CO_2	10	13.3	8.3	200 ±12	520 ±52	15.35 ±1.41	2.98 ±0.04
Air	9	9.4	7.2	88 ± 7	247 ±35	7.50 ±0.85	3.04 ±0.07
Cotton plugs	4	8.5	6.5	66 ± 5	132 ±17	4.52 ±0.55	3.44 ±0.08
Open	3	10.7	7.7	164 ±20	690 ±66	18.53 ±1.33	2.69 ±0.21
Experiment 4, agar—54 days:							
0.8 per cent CO_2	10	15.4	9.1	254 ±13	1,192 ±61	27.3 ±1.2	2.28 ±0.05
0.4 per cent CO_2	8	12.5	9.5	209 ±11	959 ±37	22.6 ±1.2	2.35 ±0.07
0.2 per cent CO_2	8	11.3	9.5	332 ±35	890 ±47	20.2 ±1.4	2.24 ±0.05
0.1 per cent CO_2	8	11.7	10.3	281 ±20	690 ±27	17.7 ±0.09	2.56 ±0.06
Air	9	9.5	10.6	136 ± 9	271 ±10	9.47 ±0.22	3.48 ±0.06
Cotton plugs	9	7.5	9.6	52 ± 6	129 ± 9	4.97 ±0.35	3.06 ±0.26

to be significant. Although the difference in the means of two adjacent series, i.e., 0.1 per cent and 0.2 per cent CO_2, or 0.4 per cent and 0.8 per cent are not always significant, it is apparent that the greater the pCO_2 in the atmosphere supplied the plants, the greater is the dry weight and the nitrogen fixed. Because of the large variation in the individual responses, this would not be apparent if only two adjacent series were compared, but when the experiment is considered as a whole there can be little doubt but that each increase in the pCO_2 resulted in an increase in the dry weight and in the nitrogen fixed. Of particular interest are the data on the percentages of nitrogen in the plants; they show that not until the CO_2 concentration reached 0.4 per cent was there

TABLE 3

Summary of the probabilities that the differences observed in the means arose from chance

Experiments 3 and 4

TREATMENT	0.1 PER CENT CO_2				0.2 PER CENT CO_2				0.4 PER CENT CO_2			
	Number nodules	Dry weight	Total N	Per cent N	Number nodules	Dry weight	Total N	Per cent N	Number nodules	Dry weight	Total N	Per cent N
0.1 per cent CO_2:												
Experiment 3					0.75	0.25	0.45	0.20	0.02	0.04	0.10	<0.01
Experiment 4					0.20	<0.01	0.15	<0.01	<0.01	<0.01	<0.01	<0.01
0.2 per cent CO_2:												
Experiment 3	0.75	0.25	0.45	0.20					0.02	0.20	0.30	0.03
Experiment 4	0.20	<0.01	0.15	<0.01					<0.01	0.30	0.35	0.15
0.8 per cent CO_2:												
Experiment 3	0.03	<0.01	0.01	<0.01	0.03	0.01	0.07	<0.01	0.75	0.25	0.30	0.20
Experiment 4	0.04	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.50	0.20	<0.01	<0.01	0.30
Air:												
Experiment 3	<0.01	<0.01	<0.01	0.40	<0.01	<0.01	<0.01	0.12	<0.01	<0.01	<0.01	<0.01
Experiment 4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

any significant change in the dry weight-nitrogen relation. This would indicate that, to a certain limit, the large increases in carbohydrate formation by the plants were accompanied by corresponding increases in the nitrogen fixed. Beyond this limit the differential between photosynthetic activities and respiration proceeded at a more rapid rate than did the nitrogen fixation process.

The plants grown in bottles closed with a cotton plug were lower in dry weight and nitrogen than were any of the rest; of especial interest is the fact that the percentage of nitrogen in these plants is much higher than it is in plants of other series, indicating that because of the low pCO_2, carbon assimilation is the limiting process in the development of plants grown in cotton-plugged containers. The data on the plants grown in the open bottles support the observation already made: if additional CO_2 is supplied to plants grown in a closed container, they are able to overcome the handicap imposed by their unnatural habitat. However, this does not mean that the cause of the poorer growth noted in closed systems is lack of CO_2, but that by stimulation of the carbon assimilation with increased CO_2, the handicaps in growth imposed on the plant by the closed container can be overcome.

Experiment 4

Experiment 4 was a repetition of experiment 3 except that the plants were grown on Crone's agar instead of sand. The cultures were planted with red clover and inoculated with *Rh. trifolii* 209 on September 13, 1931. They were allowed to grow for 17 days before being connected to the manifolds; at this time the plants had their first leaves and some nodules were formed. Soon after the various gas mixtures were started, the plants receiving additional CO_2 begin to show signs of carbohydrate excess; the stems became red, the leaves turned yellow, and it was evident that the plants were suffering for lack of nitrogen. This period lasted for about 10–15 days, then the plants suddenly recovered and in 2 weeks had filled completely the 64-ounce bottles. It was apparent that the additional CO_2 was of no benefit and was possibly detrimental to the plants unless the nodules had developed to a point at which they could supply sufficient nitrogen for the needs of the growing plant. It is significant that this period of nitrogen starvation is not nearly so apparent in plants grown in sand and supplied with increased CO_2, which probably means that there is some factor which limits the nitrogen fixation process in plants grown in agar. The plants were harvested when 54 days old; during the last 2 weeks of their growth, artificial lights were used to supplement the sunlight.

The plants receiving increased CO_2 were stocky and thrifty, dark green, pubescent, and with some reddening of the stems. The stems were heavy, the leaves very large, and the roots were short and thick. They could hardly be distinguished from plants grown in open pots. The plants receiving the higher concetrations of CO_2 were somewhat larger and showed more carbohydrate excess than did the others. The nodules on the 0.4 per cent and 0.8 per cent CO_2 series were very large and placed on both the tap and the lateral roots. There

were more nodules on the 0.1 per cent and the 0.2 per cent CO_2 series, but many of these were the small, round, scattered type. The plants supplied with air were not nearly so large as the ones receiving additional CO_2, but they were much better than those grown in bottles closed with cotton plugs. The nodules on the plants supplied with air were small to medium in size and were found on both the tap and the lateral roots; in the cotton plugged series, the nodules were few in number and located near the crown on the taproot.

The results of the analyses are given in table 2, experiment 4. The means of the variables measured were tested for significance by the analysis of variance; as in experiment 3, the populations were found to be non-homogeneous, indicating that the treatments produced a significant effect. The observed differences in the means were further tested by the modified "Student" method; the results of this test are summarized in table 3.

The results are very similar to those of the sand series; with increasing CO_2, there is an increase in size, dry weight, and total nitrogen in the plants. Although the difference between the means in adjacent series is not always significant there is a definite trend toward higher values of the variables measured as the pCO_2 supplied is increased. In general the results of this experiment are little different from those of experiment 3 but there is one outstanding difference—the percentage of nitrogen in the plants. Although both the dry weight and nitrogen fixed by the plants grown under a given pCO_2 is somewhat higher in the agar series than is the corresponding value in the sand series, the percentage nitrogen in the plants is, in general, somewhat lower. Moreover, in the case of clover grown on sand, there was no decrease noted in the percentage of nitrogen in the plants until the 0.4 per cent series was reached, but with clover grown in agar, an increase in the CO_2 content from 0.03 to 0.1 per cent resulted in a significant decrease in the percentage of nitrogen. An increase from 0.1 to 0.2 per cent in the CO_2 content of the atmosphere caused a slight decrease in the value of this figure, but further increases in the CO_2 content were without effect on the dry weight-nitrogen relation. As observed in experiment 3, the percentage of nitrogen in the plants with a carbon dioxide deficient atmosphere (cotton plugs) is higher than in any other series.

Experiment 5

The results of experiments 1 to 4 have established the importance of the photosynthetic process to nitrogen fixation by clover when grown in a closed system. The use of closed bottles for plant containers is convenient since this simplifies the control of the gas mixtures furnished the plants, but it is believed that the major findings of experiments 1–4 should be checked for the case of plants growing in the open. Accordingly, an experiment was made in which additional CO_2 was supplied clover plants which were grown in the 64-ounce bottles open to the air. The plants were placed in the greenhouse November 11, 1931, and aeration with an air-CO_2 mixture and with air alone started immediately; the rate of aeration was 100 cc. per minute per bottle. Artificial illumination was used to supplement sunlight throughout this experi-

ment. The average CO_2 content of the air inside the bottles supplied with this gas was 0.5 per cent, whereas that of the air alone was 0.033 per cent. The age of the plants at time of harvest was 48 days.

The plants in the series which received the CO_2 plus air mixture were very large, stocky, and showed some carbohydrate excess; the leaves were very large and dark green. The plants protruded 3 to 4 inches from the bottles and several were blooming. All were well nodulated, but the nodules were somewhat scattered throughout the plant system. The plants which were aerated with air alone were not quite so stocky as the series receiving CO_2 but were inclined to be tall and green. There were not so many nodules on the roots and only one plant was blooming. The plants grown in open bottles without aeration were very similar to those aerated with 0.03 per cent CO_2 (air).

TABLE 4

The effect of added CO_2 on clover plants grown in open bottles

Experiments 5 and 6

TREATMENT	NUMBER OF SAMPLES	LENGTH OF TOPS	LENGTH OF ROOTS	NUMBER OF NODULES	DRY WEIGHT	NITROGEN	
						Total	Per cent
		cm.	*cm.*		*mgm.*	*mgm.*	
Experiment 5—48 days:							
Air plus CO_2	7	....	....	.....	1,255 ±87	30.4 ±1.7	2.42 ±0.14
Air	7	....	....	.....	765 ±45	20.7 ±1.0	2.72 ±0.09
Not aerated	5	....	....	.....	587 ±38	15.9 ±0.9	2.71 ±0.04
Experiment 6—45 days:							
Air plus CO_2	7	19.7	12.0	179 ±18	1,384 ±58	34.5 ±1.7	2.49 ±0.06
Air plus CO_2 and nitrate	5	16.2	12.6	.....	1,136 ±46	23.7 ±1.5	2.08 ±0.19
Air	7	15.7	9.4	104 ± 7	762 ±35	21.8 ±0.9	2.87 ±0.04
Air plus nitrate	5	15.8	11.8	.....	780 ±21	22.7 ±0.9	2.92 ±0.13
Not aerated	6	16.0	10.0	112 ±10	747 ±32	22.7 ±1.1	3.05 ±0.12

The data, which are presented in table 4, experiment 5, confirm the results obtained in the previous experiments. The dry weights of the plants given additional CO_2 were increased about 65 per cent and the nitrogen fixed was increased about 50 per cent. As a result, the percentage of nitrogen in the plants receiving air plus CO_2 was somewhat lower than in those aerated with air, or in those merely open to the air.

Experiment 6

In this experiment, which was a repetition of experiment 5, some of the plants were supplied with combined nitrogen in order to compare their growth with plants fixing nitrogen. The plants were set up on December 13, 1931. Some were inoculated with *Rh. trifolii* 209 and others were supplied with combined nitrogen as NH_4NO_3 periodically during the growth period. An attempt was made to add the NH_4NO_3 at the start at a rate that would allow the inoculated plants to keep pace with those supplied with combined nitrogen.

After nitrogen fixation was well under way in the inoculated plants, the NH_4NO_3 was added in excess to the *nitrate series* in order to determine whether the nitrogen fixation process was limiting the growth of the inoculated plants. The addition of the NH_3NO_3 was as follows: 7 days, 2 mgm.; 15 days, 5 mgm.; 21 days, 5 mgm.; 28 days, 5 mgm.; 35 days, 10 mgm.; 38 days, 5 mgm.; 40 days, 2 mgm.; 42 days, 5 mgm.; 44 days, 3 mgm. A total of 45 mgm. of combined nitrogen per bottle was added to the nitrate series but algae grew in all of the cultures so that the actual amount available to the plants at any time was lower than the additions indicate. The plant cultures were not aerated until 20 days after planting in order to allow nitrogen fixation to start before the increased pCO_2 was supplied the plants. After 25 days of aeration, the plants were harvested.

The inoculated plants which received CO_2 were large, dark green, and showed some signs of carbohydrate excess. The nodules were predominantly large on the taproot with numerous medium and small nodules scattered on the lateral roots. The plants that were supplied with nitrate and additional CO_2 were very similar in appearance to the inoculated plants. The inoculated plants aerated with 0.03 per cent CO_2 (air) and those which were merely open to the air were somewhat smaller than the plants in the CO_2 series. They were dark green and showed no indications of carbohydrate excess. The nodules were large and located almost exclusively on the taproot. The nitrate controls of this series also had smaller plants than those supplied with additional CO_2.

The analyses are given in table 4, experiment 6. In general they confirm the results of experiment 5, i.e., an increased pCO_2 in the atmosphere supplied plants grown in the open results in a large increase of dry weight and a somewhat smaller increase in the quantity of nitrogen fixed. The nitrate controls of the plants receiving CO_2 did not assimilate as much nitrogen as was fixed by the inoculated plants, but this might have been due to the competition for the added nitrate by the algae growing in these cultures. A comparison of the effect of increased CO_2 on plants fixing nitrogen and those supplied with combined nitrogen is being made under more controlled conditions.

Experiment 7

In all of the previous experiments, red clover was used as the experimental plant. One experiment was made in which alfalfa was grown on sand in an atmosphere containing 0.2 per cent CO_2 and compared with control plants in bottles closed with a cotton plug (less than 0.03 per cent CO_2). The results were practically identical with those obtained with clover plants; the dry weight of the plants supplied with CO_2 was almost 3 times that of the controls, whereas the increase in nitrogen fixed was about 2.5 fold.

DISCUSSION

General

The results of seven experiments in which over 200 plant cultures were used demonstrated conclusively that nitrogen fixation by red clover in association

with its specific organism, *Rh. trifolii*, could be markedly increased by growing the plants in an atmosphere containing a higher partial pressure of CO_2 than that of air. Plants grown on sand plus modified Crone's solution in an atmosphere deficient in CO_2, e.g., in a cotton plugged bottle, have a high percentage of nitrogen indicating that the carbon assimilation and not nitrogen fixation is the process limiting the growth of the plant. Under these conditions the percentage of nitrogen in a plant is between 3.0 and 3.5 per cent; if plants are grown in a closed system and supplied with normal air (0.03 per cent CO_2) the percentage of nitrogen in the plant decreases to slightly less than 3 per cent, but the dry weight and total nitrogen are increased. Further increases in the pCO_2 in the atmospheres furnished the plant results in 100–200 per cent increases in both dry matter and nitrogen fixed with no change in the percentage nitrogen in the plant until atmospheres containing between 0.2 and 0.4 per cent CO_2 are used. As the CO_2 content is increased beyond these figures, there is a proportionately greater assimilation of carbon than of nitrogen so that while the absolute value of the nitrogen fixed increases, the percentage of nitrogen in the plant decreases. This is accompanied by typical signs of carbohydrate excess in the plants, i.e., reddening of the stems and leaves. Thus by increasing the pCO_2 in the atmosphere supplied to inoculated clover plants grown in a closed system the metabolism of the plant can be changed from a type in which carbon assimilation is the limiting factor in development, through a stage in which carbon assimilation and nitrogen fixation are in equilibrium, to a final stage in which nitrogen fixation becomes the factor limiting the growth.

With plants grown in agar, the situation is much the same except that any increase in the pCO_2 results in a decrease in the percentage of nitrogen in the plant, although the *absolute* quantity of nitrogen fixed is increased. That is, if the carbon dioxide content of the air supplied to plants grown in agar is only slightly increased, carbon assimilation (differential between photosynthesis and respiration) takes place at a more rapid rate than does nitrogen fixation. With plants grown in sand, this effect is not observed until the CO_2 content of the atmosphere reaches about 0.4 per cent. The cause of this difference is not readily apparent but it is likely concerned with the lowered gas exchange of the roots of plants in agar. If this lowered gas exchange causes a deficiency in the supply of oxygen to the nodules, less respiration will take place with consequent increase in the differential between photosynthesis and respiration; also, the deficiency in oxygen may be a limiting factor in the fixation process as suggested by Thornton (22). It is likewise possible that poor gas exchange affects the nitrogen fixation process of plants in agar by a decrease in the pN_2 in the nodule; or by an accumulation of toxic products, including CO_2, in the nodules caused by the accelerated respiration of the bacteria in the presence of an increased supply of carbohydrate.

A second effect of increasing the pCO_2 available to clover plants fixing nitrogen is the changes induced in the number, size, and distribution of the nodules.

Plants grown in a CO_2-enriched atmosphere show 2 to 3 times as many nodules as the controls in air, and the size is greatly increased; in some cases the increased size appears almost pathological. However, more striking than either the increase in the number or size of the nodules is the change in distribution. Ordinarily the nodules are clustered around the crown on the taproot, but as the pCO_2 in the atmosphere rises there is a tendency to invade the lateral roots, so that plants grown in relatively high concentration of CO_2 (0.5 per cent) have a root system in which the nodule distribution is very similar to that of plants inoculated with poor strains of the organisms. This development of nodules on the secondary roots suggests that possibly invasion of these roots takes place in all plants, but because of limited carbohydrate supply, the organisms in the lateral roots are kept from developing and no nodules appear. A poor strain would then be an organism in which parasitism had been accentuated to a degree that it could compete with the plant for its carbohydrate even when this is present at a low level in the plant sap. On the other hand, the invasion of the lateral roots on plants high in carbohydrates may mean only that the chemotaxis of the invasion of roots is related to the carbohydrate metabolism of the plant.

Theoretical

The results of the experiments described in the text throw some light on the mechanism of the fixation process by the association of the plant and bacteria. Because of the inability to demonstrate fixation by the bacteria apart from the host plant, there has been engendered some doubt as to whether or not the bacteria are directly concerned in the fixation process. The picture presented by plants receiving additional CO_2 suggests that the bacteria are the actual agents concerned in the fixation. The period of lag during which the plant nearly succumbs because of the lack of nitrogen is suggestive of the development of bacterial tissues in the nodule independent of the plant activities. If the plant were the primary agent concerned in the fixation, it does not appear likely that its carbon assimilation and nitrogen fixation functions would be so completely independent that there could occur such a displacement of the carbon-nitrogen equilibrium as is observed. On the other hand, if the bacteria are the causal agents in the fixation but are dependent on the plant for a source of energy, the nature of the stimulative effects of increased photosynthesis by the plant becomes apparent. Another observation that tends to link the bacteria with the actual fixation process is that made on the size and number of the nodules. In those plants which developed a large number of nodules when supplied with additional CO_2, the size of the nodules formed was not greatly increased; other plants responded to CO_2 treatment by an increase in the size of the nodules rather than an increase in the number. Thus it appears that the need for additional nitrogen is met by either development of new centers of nitrogen fixation, or by increased activity on the part of the centers already present. This evidence of a correlation in the development

of the bacteria in the plant with the quantity of nitrogen fixed, indicates that the function of the bacteria in the process is something more than a mere stimulation of the plant. Examination of the nodules on plants supplied with increased CO_2 shows that they are filled with bacteriods much larger than those found in nodules of plants supplied with air alone. It is planned to make cytological studies of these nodules in an effort to obtain more information in regard to the effect of the increased supply of carbohydrate on the bacteriods and nodular tissue.

There is still to be answered the question in regard to the specific nature of the observed stimulation of the nitrogen fixation process by an increase in the carbon assimilation of the plant. The obvious answer is that the increase in the rate of photosynthesis gives rise to a higher level of carbohydrate in the sap of the plant, which furnishes the bacteria with the energy necessary for an increase in the fixation of nitrogen. Although this sounds plausible, it is possible that the function of the plant is to furnish not merely a source of energy but specific compounds, intermediate in its carbohydrate metabolism, which are required by the bacteria by reason of the mechanism chosen for the fixation process. For example, if the first compound formed in the fixation is the result of an equilibrium reaction, the fixation process is conditioned by the removal of this compound. This might be done by combination with some specific intermediate of the carbon assimilation process, and in such a case the concentration of this intermediate in the plant would be the factor limiting the fixation of nitrogen by the bacteria.

Practical application

The discussion has dealt exclusively with the theoretical aspects of the results of the experiments reported, but equally important are the practical applications to agricultural science. In view of the marked stimulation of nitrogen fixation by clover and alfalfa plants supplied with additional CO_2, it might prove decidedly advantageous for the practical agriculturist to adopt methods of soil culture which will cause an increase in the pCO_2 in fields cropped to these leguminous plants. Although the use of commercial sources of CO_2 is probably impractical, the application of organic manures and the necessary cultivation for maximum activity of soil microorganisms would very likely prove a most economic investment in carbon dioxide production. Field experiments in which the possibility of forcing an increased synthesis of nitrogen by leguminous plants through application of various types of CO_2 manuring might prove of decided value to agricultural science.

A second possible application of these data is to those experiments in which nodule production is used as a criterion of the efficiency of a culture of rhizobia, e.g., the testing of commercial cultures. Ordinarily, this testing is done on a sterilized sand substrate in which carbon dioxide production is at a minimum, whereas the cultures are intended to be used in soils producing appreciable quantities of CO_2. In view of the results on the influence of pCO_2 on the num-

ber of nodules, it might be profitable to compare cultures under conditions such that the carbohydrate metabolism of the plant can not be the limiting factor in the production of nodules.

SUMMARY

A review of the literature which deals with the effects of supplying additional CO_2 to plants, indicate that leguminous plants are especially responsive to an increase in the pCO_2 of the atmosphere in which they are growing. The relation of this observation to the fixation of atmospheric nitrogen by the associated growth of these plants with bacteria has been investigated.

A method has been developed for the estimation of sand adhering to the roots of plants which are to be used for the determination of total nitrogen. It consists of digesting the plant material by the usual Kjeldahl procedure, diluting with 250 cc. distilled water, then comparing with suitable "sand standards." These standards are prepared from sand-free plant material to which is added a known weight of sand. The maximum error in the determination is 10 to 15 per cent; with this accuracy, the error in the estimation of the dry weight of plants is less than 2 per cent.

Inoculated clover plants were grown on agar and sand in a closed system and provided with atmospheres which contained 0.03, 0.1, 0.2, 0.4 and 0.8 per cent CO_2. The effect of the different pCO_2 on the general growth and nitrogen fixation was determined by suitable measurements. In general as the pCO_2 in the atmosphere is augmented, the leaf area, length of tops, stockiness of plant, excess of carbohydrate, and number of nodules formed are increased. Raising the CO_2 content of the atmosphere from 0.03 (air) to 0.1 per cent results in a pronounced increase in these characteristics of plant growth, but further increments in the pCO_2 do not induce correspondingly greater development.

Clover plants, grown in agar and which were supplied with atmospheres containing 0.1 per cent or more CO_2, show an increase in both the dry weight and the quantity of nitrogen fixed, when compared with the controls supplied with air. However, the gain in nitrogen is relatively less than that in dry weight, and this difference causes the plants receiving additional CO_2 to have a lower *percentage of nitrogen* than the controls. With plants grown in sand, the lag in nitrogen fixation as compared with carbon assimilation does not occur until the CO_2 content of the atmosphere is raised to about 0.4 per cent. This difference between plants in agar and in sand, when furnished with small increments of CO_2, is believed to be related to the lower rate of gas exchange of the roots of plants in agar.

In addition to an increase in the number of nodules, addition of CO_2 results in a change in the size and distribution. Plants supplied with the higher partial pressures of CO_2 often exhibit nodules which are enormous in size, especially those located on the taproot. Invasion of the lateral roots also occurs so that the distribution of nodules on the root system becomes definitely similar to that associated with infection by poor strains of the organism.

The major findings, obtained with clover plants in a closed system, have been verified for clover planted in bottles open to the air and for alfalfa grown in the closed system.

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BOOK REVIEWS

Root Nodule Bacteria and Leguminous Plants. By EDWIN BROUN FRED, IRA LAWRENCE BALDWIN, AND ELIZABETH MCCOY. University of Wisconsin Studies Number 52, Science No. 5. The University of Wisconsin, Madison, Wisconsin, 1932. Pp. XXII + 343, pl. 46, charts 4; paper cover. Price $3.

This is a comprehensive monographic treatise covering the numerous phases of research concerned with legume bacteria and legumes. Chapter headings will suggest the nature of organization of the material: 1. The history of leguminosae in agriculture (ancient use of legumes and discovery of bacterial association). 2. General description of the leguminosae, their distribution and importance (includes composition). 3. The occurrence of root nodules (discovery, importance, characteristics). 4. The isolation and study of the root nodule bacteria (methods, occurrence, contaminants in the nodule). 5. The morphology and life cycle of the root nodule bacteria. 6. Cultural and biochemical characteristics of the root nodule bacteria (nutrition, nitrogen-fixation, action of bacteriophage, composition of the bacteria). 7. Some factors which influence the growth and longevity of the nodule bacteria (environmental conditions as oxidation-reduction, aeration, temperature, desiccation, sunlight, reaction, organic and inorganic substances). 8. Species relationships (classification and differentiation of species, cross inoculation groups, nomenclature). 9. The formation of nodules, their histology and cytology. 10. Relationship between leguminous plants and bacteria (strain variation, physiological efficiency, parasitic strains of the bacteria, mechanism of nitrogen fixation). 11. Factors that influence nodule production (environmental conditions and nodule formation—aeration, moisture, light, temperature, reaction, inorganic salts, nitrogenous substances, organic materials). 12. Economic importance of leguminous crops (gains in nitrogen by growth of legumes, association of legumes and non-legumes). 13. Natural and artificial inoculation (methods of inoculation—soil and pure culture methods). There follows an appendix in which is discussed the preparation, distribution, and testing of artificial cultures of root nodule bacteria. There are a complete list of references and an index.

In soil microbiology there is scarcely a chapter which is more impressive than that dealing with the nodule bacteria. This is partly accounted for by the outstanding achievements of an illustrious body of investigators who have worked in the field and partly by the immense practical benefits which have followed the application of principles established in these researches.

The voluminous literature on legume bacteria bears witness to the intense interest in the subject.

Fifty years of probing have brought forth reports in technical journals, bulletins and whole volumes in English, German, French, Italian, Dutch and the Scandinavian languages, to mention only the most common sources of material upon the Leguminosae. So much study in a comparatively narrow, though vital and perplexing field have often quite naturally "—resulted in unconsciously going over the ground already known and recorded by different investigators."

A project which aims to gather together the published reports of such a subject, to organize it, and to present the details comprehensively and impartially is undoubtedly meritorious. It is doubtful whether any persons are better qualified to undertake this work than the authors, long experience with numerous ramifications of the subject enabling them to speak with authority and to judge debatable points keenly.

Even a casual examination indicates that the volume is excellently arranged; more detailed examination reveals the care and thoroughness with which the authors present the facts. They have preserved a fine balance in the material, eliminating any over emphasis of their own extensive researches. The plates are excellent.

The far-reaching influences of the monograph are difficult to estimate. It seems inevitable that it should accomplish the authors' desire to avoid repetition and focus attention on fruitful unexplored branches of research. It likewise appears to have value no less important in correcting false or uncertain opinions which appear in discussions of legumes and legume bacteria.

It is hoped that others will be inspired to do as much for various branches of soil microbiology.

ROBERT L. STARKEY.

Fixed Nitrogen. Edited by HARRY A. CURTIS. American Chemical Society Monograph Series. The Chemical Catalog Company, Inc., New York, 1932. Pp. 517, figs. 82.

The book is one of a series of scientific and technologic monographs published under the auspices of the American Chemical Society. In the General Introduction, the board of editors of the American Chemical Society say: "Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line." They say further: "The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended."

In outlining the scope of the book, the editor states; "The present monograph presents first a brief survey of the general situation with regard to the distribution of the earth's total nitrogen and of the methods by which the free nitrogen of the atmosphere is made available in combined or 'fixed' form."

The book contains 16 chapters, pp. 11–490; a bibliography, pp. 491–506, and author references, pp. 507–513. There are 4 chapters by Curtis, viz: I. General Survey of the Sources and Utilization of Fixed Nitrogen: III. The Chilean Nitrate Industry: IV. Fixed Nitrogen Recovered in Coal Carbonization; and V. A History of Nitrogen Fixation Processes. Chapter II. Nitrogen Fixation by Living Organisms, was contributed by F. E. Allison; Chapter VI. Physical Methods of Studying Chemically Active States of Gases and of Investigating Catalyst Surfaces, by C. H. Kunsman; Chapter VII. The Arc Method of Nitrogen Fixation, by Norman W. Krase; and Chapter VIII. Synthetic Ammonia, by P. H. Emmett. Chapter IX. Some Physical Properties of Hydrogen, Nitrogen, Methane, Ammonia, Carbon Monoxide and Carbon Dioxide and Their Mixtures, is by Richard Wiebe; Charter X. High Pressure Equipment and Technic, by J. R. Dilley and W. L. Edwards; and Chapter XII. The Alkali Cyanide Method of Nitrogen Fixation, by E. W. Guernsey. Three chapters were contributed by H. J. Krase, viz: XI. The Cyanamide Method of Nitrogen Fixation, XII. Synthesis of Urea, and XIV. Oxidation of Ammonia. Chapter XV. Synthetic Nitrogenous Fertilizers, was written by William H. Ross and Albert R. Merz, and Chapter XVI. Nitrogen Statistics by, P. E. Howard.

The contributors have furnished us with a long-range view of the world nitrogen resources. They have also supplied us with a historical record on the development of nitrogen-fixation processes, aside from an adequate discussion of the nature of the processes themselves. The work is a valuable addition to present-day reference material on the subject of fixed nitrogen.

Methoden für die Untersuchung des Bodens. Part I. Edited by O. LEMMERMANN. Verlag Chemie, Berlin, 1932. Pp. 90, ill. 2.

As is indicated by the title, the volume is devoted to a discussion of methods for the study of soils. It was prepared in behalf of the German agricultural experiment stations and the German Society of Soil Science. The five divisions of the book deal, respectively, with: A. the sampling of the soils, B. the physical examination of mineral and humus soils, C. the examination of mineral soils, D. the examination of peat soils, and E. examination of forest soils. The section devoted to sampling, outlines methods that would best serve the purpose of those interested in soil mapping and classification as well as in the study of physical, chemical, plant physiological, and microbiological attributes of soils. Methods are also described for the taking of samples of peat soils and of samples of soil profiles.

The second section concerns the mechanical analysis of soils and the determination of specific gravity, water-holding capacity, heat and wetting, and hygroscopicity. Section 3 is divided into three parts, dealing respectively with the chemical examination of soils, the determination of the fertilizer requirements, and microbiological studies. Methods of determining total and so-called available plant-food are given and likewise older and more recent

methods which concern the calcium status of soils and the determination of so-called available nutrients by plant physiological and chemical methods. Among the methods described are those of Mitscherlich, Neubauer, Schneider, König Hasenbäumer, Lemmermann, Fresenius, and 'Sigmond. There is a reasonably complete description of methods dealing with the determination of microbiological properties of soils. The same may be said also of section D, where methods for the study of peat and muck soils are described. No methods are specifically given for the examination of forest soils. The editor suggests that until such methods are more fully established those given for soils in general be employed.

The book, which is really a laboratory manual, will be found useful by students of soils, who will no doubt appreciate both the text and the reference to the appropriate literature.

Land Drainage. By W. L. POWERS AND T. A. H. TEETER. Second Edition, Revised and Enlarged. John Wiley & Sons, Inc., New York and London, 1932. Pp. x + 353, figs. 169.

The book is divided into four parts, dealing respectively with field drainage, district drainage, special drainage problems, and drainage surveying. There is also an appendix, which deals with farm drainage laboratory exercises, tables for use in Kutter's Formula, and U. S. Census of Drainage—1930.

In the Foreword of the first edition—which appeared in 1931, the author, W. L. Powers, pointed out that the "volume is intended, first, as a text book for students of general agriculture or agricultural engineering; second, as a reference book for practical farmers; and third, as an aid to owners of wet, overflowed, marsh, swamp or alkaline land who desire to improve their holdings." In the Introduction to the second edition, the senior author notes that "During the decade which has passed since the first edition was prepared, a number of notable developments have occurred in the drainage field. River and flood control have required attention of the national congress; soil erosion and terracing has become a problem of national importance; war salvage explosives have been extensively used in drainage and clearing; drainage in irrigated sections by means of pumping from wells has found wide application; progress has been made in developing pumping and excavating machinery, in building ditches with the aid of a scarifier in connection with running water, and in the use of vertical drainage."

Aside from the technic of field drainage dealt with in Part 2, there is a very helpful discussion of drainage districts and drainage laws, of flood control, and of allied topics. Among the special drainage problems, the authors discuss the drainage of tidal and overflowed marsh lands and of irrigated lands, and likewise drainage by means of pumping wells, prevention and control of erosion, with special reference to terracing. Part 4 deals with drainage surveying and practices.

The book will be found useful as a text on land drainage as well as a reference book for teachers and land owners.

Der Einfluss der Handelsdünger auf das Pflanzenwachstum und auf verschiedene Eigenschaften Kalkarmer Mineralböden. By LUDWIG SCHMITT with an introduction by HUBERT RÖSSLER. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, 1932. Pp. 186, ill. 12, graphs 20.

The rapid expansion in the manufacture of synthetic ammonia has resulted in an increased consumption of ammonia salts. This increase in consumption has been particularly noteworthy in Germany. As a result of the continued use of ammonium sulfate and of other ammonia salts, soil acidity has become so pronounced in some sections of Germany as to have led to the studies recorded in this book. The volume is divided into six sections, dealing respectively with an introductory statement; the physiological reaction of potassium salts; the physiological reaction of phosphorus fertilizers; the physiological reaction of nitrogen fertilizers; the action of potash fertilizers as indicated by long-continued field experiments at the Darmstadt Station; and the determination of the lime requirements of soils. There is also a concluding paragraph by the author and a reference list containing 86 items.

Fertilizers and Crop Production. By L. L. VAN SLYKE. Orange Judd Publishing Company, Inc., New York. 1932. Pp. xiv + 493, frontispiece, ill. 70, tables 63, appendix.

The present volume represents a revision of "Fertilizers and Crops" by the same author, published in 1921. It is stated by the author in his preface: "In the preparation of this book, it has been the author's aim to keep in mind not only the requirements of students in agricultural colleges and high schools but as well the needs of student farmers who, in increasing numbers, are directly and vitally concerned in the profitable production of crops. The book can be used to advantage in the educational work promoted by granges and other farmers' organizations."

The five parts of the book deal respectively with factors of soil fertility; functions and physical properties of soils; sources and composition of materials used as fertilizers; factors that guide the selection of fertilizer materials; and practical use of fertilizers in growing individual crops. Like its predecessor, this book is extremely valuable in supplying the teacher, research worker, and extension specialist with extensive and authoritative information on the composition of soils, fertilizers, and plants. Beyond that, there is a sound and wholesome philosophy which finds expression. This may perhaps be best illustrated by the following statement of the author. "Generally speaking, the fundamental purpose of this book is to study the conditions under which plant-foods, whether in the form of soil compounds, of farm-produced materials, or of commercial fertilizers, can be conserved and at the same time utilized with the greatest efficiency and economy in the production of crops."

Soils. Their Origin, Constitution, and Classification. By GILBERT WOODING ROBINSON. Thomas Murby & Co., England, and D. Van Nostrand Co., New York. 1932. Pp. xv + 390, frontispiece, figs. 12, tables 38.

The author, who is professor of agricultural chemistry in the University College of North Wales, has been for many years a careful student of the genesis, formation, classification, and mapping of soils. As the author himself notes: "I have written primarily for those who are interested in the soil as an object of study in itself, and secondarily for those whose interest lies in its economic or geographical significance; but it may be hoped that the book will prove of value to botanists because of the importance of the soil in ecology, and to geologists because of the part played by the soil in the weathering cycle."

The main body of the book consists of 19 chapters and an appendix devoted to methods of analysis. The designations of its several chapters are: Introductory; General View of the Constitution of the Soil; The Pedogenic Processes; The Clay Complex; Base Exchange and Other Reactions of the Colloidal Complex; Soil Organic Matter; General Physical Properties of Soils; Water Relationships of Soils; Soils of the Podsolic Group; Tshernosems and their Related Groups; Ground-water Soils, Including Peats; Saline, Alkaline and Soloti Soils; Soils of the Humid Tropics and Subtropics; Soils Associated with Calcareous Parent Materials; The Classification of Soils; The Geography of Soils; Soil Surveys; Soil Analysis; Soils, Plant Growth and Agriculture.

The author is to be commended for his effort to establish a wider basis of soil classification and mapping, and to create a clearer conception of soil factors in relation to world agriculture. This work will be welcomed by the teacher and students of soils and should find a place in every modern library.

pH and its Practical Applications. By FRANK L. LAMOTTE, WILLIAM R. KENNY, AND ALLEN B. REED. The Williams & Wilkins Co., Baltimore, Md. 1932. Pp. vii + 262, charts 18, figs. 15, tables 27.

"In this work," say the authors, "we have endeavored to set forth the fundamental principles of hydrogen ion control in a manner that would prove helpful to the operating chemist. No attempt has been made to present a discourse on the scientific phases of hydrogen ion measurements, since this has already been covered by eminent authors. We have endeavored to bear in mind the desires of the practical technologist, who is aware of the value of pH control, but who merely wishes a working knowledge of its application, rather than a thorough treatise of the subject as a pure science."

Section A of the book is entitled "Mechanism of Hydrogen Ion Determinations" and contains chapters on introduction and preliminary discussion; hydrogen and hydroxyl ions; buffer action; determination of pH; sources of error. Section B deals with "Practical Application of Hydrogen Ion Determinations in Industry and Science," and contains chapters on municipal and industrial water supply; water corrosion problems; disposal of sewage and industrial waste; sugar industry; gelatin and glue; leather manufacture; textile industry; pulp and paper industry; food industries; cleaning processes; electrodeposition of metals; general industrial chemistry; bacteriology, pathology and titration processes; and soils.

The authors have rendered a distinct service to laboratory workers by bringing together in readily usable form information on hydorgen ion determinations and appropriate equipment for such determinations.

Der Kationen- und Wasserhaushalt des Mineralbodens. By P. VAGELER. Verlag von Julius Springer, Berlin, 1932. Pp. vi + 336, ill. 32, chart 1.

The principal portions of the book deal with the outlining of problems and objectives, the physical, chemical, and biochemical processes in soils; and methods of research and analysis. There are a comprehensive bibliography of 398 items and author and subject indexes. A large chart at the end of the book is entitled: "Übersichtstabelle wichtiger Bodentypen der Erde in Lösungs- und Komplexbau."

The author notes in the preface that soil science as an independent science is relatively new, and that it has not been sufficiently rounded out to reflect fully world-wide conditions and relations. He notes, further, that there still exist soil science teachings distinctly national in character. There are often conflicting views and teachings, and, despite the influence of the International Society of Soil Science, there are but few teachings and conclusions which find acceptance beyond a small group of specialists. It is gratifying to find that this book, together with some other recent treatises, has contributed in a substantial way toward giving us a broader outlook on the field of soil science.

The Geology and Water Resources of Northwestern Minnesota. By IRA S. ALLISON. The University of Minnesota Press, Minneapolis, 1932. Pp. xii + 245, tables 96, figs. 36.

The book deals primarily with the physiography of the area, general geology and yield of water, and chemical character of the waters. Beyond that, water supplies of the 26 counties are considered by the author.

The book is a distinct contribution to the body of knowledge on water supplies and their character. The significance of the information contained in the book is well indicated by the data given in the several tables. For instance, Table I is entitled "Average of All Dissolved Solids in Waters of Northwestern Minnesota by Counties"; Table II, "Relative Salinity of Waters from Different Sources in Northwestern Minnesota"; and Table III, "Average Composition of Waters from Streams and Lakes of Northwestern Minnesota." The following tables contain original data of a similar character. In view of the great importance of water supplies, their sources, composition, pollution, and treatment in our complex economic and social life, the author deserves recognition for the information which he has collected and systematized.

JACOB G. LIPMAN.